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SIMON FLEXNER, M.D.

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ETIOLOGY OF YELLOW FEVER.

VII. Demonstration of Leptospira icteroides in the Blood, Tissues, and Urine of Yellow Fever Patients and of Animals Experimentally Infected with the Organism.

By HIDEYO NOGUCHI, M.D.

(*From the Laboratories of The Rockefeller Institute for Medical Research.*)

(Received for publication, May 16, 1919.)

The number of *Leptospira icteroides* found in the blood of yellow fever patients was so small that a prolonged examination of blood specimens was necessary to discover one organism, even when positive transmission had been obtained by injection of the blood into guinea pigs. In most cases the organism could not be detected in the blood, perhaps owing to lack of time. To devote much time to microscopic examination during the period while the work with yellow fever patients was being carried on was impossible and also inadvisable, since it was a matter which could be taken up at a later date when more time was available. Hence, what will be reported in this paper is not final but is the preliminary account of what was accomplished under the circumstances. The demonstration of the organism in the blood and various organs of animals experimentally infected with *Leptospira icteroides*, on the other hand, was much more satisfactorily accomplished. The infection could be produced at the desired time and the material obtained in sufficient quantity at any stage of the disease.

Examination of Material from Yellow Fever Cases.

As already stated[1] a minute organism belonging to the genus *Leptospira* has been found both in the blood and in the tissues of various organs, particularly the liver and kidneys, of guinea pigs

[1] Noguchi, H., *J. Exp. Med.*, 1919, xxix, 565.

which contracted an experimental infection after being inoculated with the blood of yellow fever patients taken during the early stages of the disease. In three instances (Cases 1, 4, and 6) the organism was found in the cultures made directly from the blood of yellow fever patients. A special effort was made, therefore, whenever a case of yellow fever was admitted to the hospital or an autopsy was performed, to find the same organism in the blood and tissues, and if possible also in the urine.

The dark-field microscope was used for examination of the fresh material. Films and sections were stained by Giemsa's or Wright's stain, by Levaditi's or Noguchi's silver impregnation methods, as occasion demanded. Fontana's silver impregnation method was applied several times without satisfactory results. Blood films were usually fixed with methyl alcohol,[2] while the impression films of various tissues from autopsies were first fixed over osmic acid vapor for 1 minute and then in absolute alcohol for 30 minutes. They were stained with Wright's stain for 30 minutes and then with Giemsa's stain over night. Six to twelve were prepared from each specimen of blood or tissue for examination.

Of twenty-seven cases of yellow fever in only three (Cases 3, 4, and 14) was the leptospira demonstrated in the blood under the dark-field microscope, and in each instance the number of the organisms found was so small that once they had passed out of the field they were not easily encountered again. The blood of two of these three patients (Cases 3 and 4) also yielded positive transmission into guinea pigs.[1] The organism was found in a few of the stained film preparations, but these will have to be repeatedly examined. Some specimens of yellow fever blood, however, were infectious for guinea pigs even when in the fresh state no leptospira could be demonstrated under the dark-field microscope (Cases 1, 2, 5, and 6). That there must have been a very small number of the organisms in such specimens is shown by the fact that the blood and organs of the infected animals contained the organisms. A negative microscopic examination of the blood indicates either absence or scarcity of the organism. Nor does the failure of the blood to reproduce a fatal experimental

[2] For the purifying of the methyl alcohol for this purpose I am indebted to Dr. Herman Edward Redenbaugh of the Commission.

infection in guinea pigs prove the absence of the organism in the specimen, since variations in its pathogenicity for guinea pigs are considerable (Case 14).

Examination of various organs under the dark-field microscope has yielded so far only one positive finding, that in the liver of Patient J. Co. (Case 4), who died on the 4th day of the disease. The liver was excised from the body (a partial autopsy) a few hours after death while the body was still quite warm. The kidney failed to show any leptospira. In the stained specimens of the blood, liver, and kidney a small number of leptospiras was demonstrated. Both the blood and the liver emulsion of this patient yielded a positive transmission of the disease to guinea pigs.[1]

A careful search for the organism was carried out with the films made from the liver, kidney, lungs, adrenals, mesentery, and inguinal glands from ten more cases, with so far a positive finding in the liver of one case (Case 5). Some of these slides were poorly stained and will have to be repeatedly examined when more time is available. In a later paper will be recorded the results of the examination of sections of the organs from patients who died of yellow fever.

Specimens of urine from twenty-one cases of yellow fever were examined under the dark-field microscope, but no leptospira was encountered. The examinations were made during the height of the illness, which is usually the 5th or 6th day of the disease, as well as during convalescence, towards the end of the 2nd week. This part of the investigation was much handicapped by the lack of a powerful centrifuge to concentrate the urine, and it will have to be repeated under more favorable conditions. As might be expected, *Treponema minutum*[3] and *Treponema calligyrum*[4] were occasionally seen in the samples of urine. A doubtful result was obtained in a guinea pig inoculated with 10 cc. of the urine from a convalescent (Case 51) on the 15th day of the disease, although no leptospira was found in the specimen.

[3] Noguchi, H., *J. Exp. Med.*, 1918, xxvii, 667.
[4] Noguchi, H., *J. Exp. Med.*, 1913, xvii, 89.

Examination of Material from Experimentally Infected Animals.

Experiments were undertaken to determine the approximate time when the blood, liver, and kidney of guinea pigs infected with the yellow fever virus first contain enough organisms to produce the infection when transferred to a normal guinea pig, and when they cease to be infective. These points are more of academic interest, as they do not affect the present sanitary measures with regard to isolation of the patients before, during, and after the actual sickness.

When a minute quantity of the virus is introduced into the subcutaneous tissue of the guinea pig no local reaction follows. The blood becomes infective as early as 48 hours after the inoculation in some instances, but more constantly so after 72 hours. The liver and kidney become infective simultaneously with the blood. The amount of blood effective for infection is comparatively large, at least 1 cc. being required during the earlier period. The demonstration of the organism in the blood is almost hopeless and has not been successful until the 5th day after inoculation, when occasional specimens have been found after a long search. The organisms are more readily found in the emulsions of the liver or kidney, sometimes more numerously in one and sometimes in the other. After the onset of the disease the number of organisms gradually increases for a time, both in the blood and in the liver and kidney. Then the relation of the organism to the blood on the one hand and to the liver and kidney on the other seems to show a certain difference. The organisms continue to increase in the organs somewhat longer than in the blood. In fact, the number of organisms in the blood becomes fewer as the disease progresses, and only a few can be detected when the jaundice and other symptoms have fully developed; that is, a day or two before death. This has been the rule with the majority of the guinea pigs in the present experiments, but there were exceptions in which the organisms were quite numerous until death. Although they increase in the liver and kidney in greater number and for a longer period, they have been found in most instances finally to disappear also from these organs. The disappearance is sudden and usually occurs about 24 hours before death, although there are instances in which they can be found in large numbers at death, sometimes

more abundantly in one or the other organ. Although it is true that when the blood contains the leptospira at the time of death the liver and kidney also contain it, yet the reverse is not always the case, the organism being found in the organs but not in the blood. An instance has not been met in which the leptospira was present in the blood but not in the liver or kidney or both.

The mechanism of the disappearance of the leptospira in so many typical experimental guinea pigs during the later stage of the infection is difficult to explain, but it may be partly due to the formation of certain still undetermined metabolic products brought about by the disorganization of the liver or kidney or some other organs. The leptospira is highly sensitive to the dissolving action of bile salts *in vitro*, and it is not inconceivable that a predecessor or a derivative of taurocholic, glycocholic, or cholic radicals in a certain stage may exert a powerful destructive action upon it.

With regard to the question of the infectivity of the blood and organs in the later stage of the infection, the statement may be made that in all instances in which the organism was seen, whether in the blood or in the organs, the material was always infective. On the other hand, in a considerable number of instances in which the dark-field search for the organism failed, no infection could be induced in normal guinea pigs by inoculation of the material. It may be supposed that the organisms had completely disappeared from the body in these cases. However, as was to have been expected, there were many instances in which, notwithstanding an unsuccessful microscopic search for the organism, inoculation of the liver or kidney, or both, but not the blood, gave rise to a typical infection in further passage, with the reappearance of the leptospira in the new host. In one instance the leptospira was microscopically detected on the third passage. It may be concluded, then, that in experimental infection in the guinea pig the specific organism survives longer in some of the infected hosts than is assumed to be the case in yellow fever in man.

SUMMARY.

Examinations of fresh blood from yellow fever patients by means of the dark-field microscope, made in more than twenty-seven cases,

revealed in three cases the presence of *Leptospira icteroides*. In no instance was a large number of organisms found, a long search being required before one was encountered. The injection of the blood into guinea pigs from two of the three positive cases induced in the animals a fatal infection, while the blood from the third positive case failed to infect the guinea pigs fatally. Careful but by no means exhaustive dark-field searches for the leptospira with fresh specimens of blood from the remaining cases of yellow fever ended without positive findings, although four of the specimens, when injected into guinea pigs, caused a fatal leptospira infection.

Stained blood film preparations from the corresponding cases were also examined, but the percentage showing the leptospira in the blood was no greater than that found by examination in the fresh state with the dark-field microscope. In fact, owing to the defective stains that were available at the time of the investigation a great many slides did not take the proper coloration with Giemsa's or Wright's stain and could not be relied upon.

Regarding the presence of *Leptospira icteroides* in various organs both dark-field and stained films were examined. In only one instance so far a few organisms were detected in the emulsion of liver taken shortly after death from a case dying on the 4th day of yellow fever. This part of the work will be reported later upon completion.

Examinations of the urine from different cases of yellow fever were made both by dark-field microscope and by inoculation into guinea pigs. The results were totally negative in thirteen cases, including many convalescents, but in one case one of the guinea pigs inoculated with 10 cc. of the urine came down on the 15th day with suggestive symptoms (suspicion of jaundice, and some hemorrhagic and parenchymatous lesions of the lungs and kidneys). This specimen showed no leptospira by dark-field examination.

In experimental infection of guinea pigs with *Leptospira icteroides* the blood became infective in many instances 48 hours after inoculation, and was always infective after 72 hours. The liver and kidney become infective simultaneously with the blood. Detection of the organism by means of the dark-field microscope has seldom been accomplished before the 5th day. The organisms are most abundant on the 6th to the 7th day, but become fewer or completely

disappear before death. In the meanwhile the number of organisms increases in the liver and kidney, from which they disappear as the jaundice and other symptoms become aggravated. When death occurs these organs seem to have lost most of the leptospira, and positive transfer by means of them is less certain. At the later stage of the disease the blood is often free from the organisms and ceases to be infective. Positive transmission with blood obtained from moribund animals is not impossible, however, even when no leptospira can be detected under the dark-field microscope.

ETIOLOGY OF YELLOW FEVER.

VIII. Presence of a Leptospira in Wild Animals in Guayaquil and Its Relation to Leptospira icterohæmorrhagiæ and Leptospira icteroides.

By HIDEYO NOGUCHI, M.D.

(*From the Laboratories of The Rockefeller Institute for Medical Research.*)

(Received for publication, May 16, 1919.)

Leptospira icterohæmorrhagiæ was demonstrated in the kidneys of wild rats first by Ido and his associates in Japan,[1] then by Stokes, Ryle, and Tytler,[2] Courmont and Durand,[3] Martin and Pettit,[4] Renaux,[5] Coles,[6] Monti,[7] Grasso,[8] and Dalmau[9] in Europe; and by Noguchi[10] and Jobling and Eggstein[11] in North America; by Nicolle and Lebailly[12] in Tunis, and by Lhéritier[13] in Algeria. That the leptospira found in wild rats is in all probability identical with that which produces infectious jaundice in man was the conclusion of Ido and his associates[1] and later of the writer[10] from the reciprocal immunity reactions between the human and rat strains. Just how the rat strain enters the human body is still a

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[1] Ido, Y., Hoki, R., Ito, H., and Wani, H., *J. Exp. Med.*, 1917, xxvi, 341.

[2] Stokes, A., Ryle, J. A., and Tytler, W. H., *Lancet*, 1917, i, 142.

[3] Courmont, J., and Durand, P., *Bull. et mém. Soc. méd. hôp. Paris*, 1917, xli, 115.

[4] Martin, L., and Pettit, A., *Compt. rend. Soc. biol.*, 1917, lxxx, 10, 574; 1918, lxxxi, 697.

[5] Renaux, E., *Compt. rend. Soc. biol.*, 1917, lxxx, 405.

[6] Coles, A. C., *Lancet*, 1918, i, 468.

[7] Monti, A., *Boll. Soc. med.-chir. Pavia*, 1917, cited by Martin, L., and Pettit, A., Spirochétose ictérohémorrhagique, Monographies de l'Institute Pasteur, Paris, 1919.

[8] Grasso, G., *Pathologica*, 1918, x, 8.

[9] Dalmau, M., *Treballs Soc. Barcelone*, 1918, cited by Martin, L,. and Pettit, A,. Spirochétose ictérohémorrhagique, Monographies de l'Institute Pasteur, Paris, 1919.

[10] Noguchi, H., *J. Exp. Med.*, 1917, xxv, 755.

[11] Jobling, J. W., and Eggstein, A. A., *J. Am. Med. Assn.*, 1917, lxix, 1787.

[12] Nicolle, C., and Lebailly, C., *Compt. rend. Soc. biol.*, 1918, lxxxi, 349.

[13] Lhéritier, A., *Bull. Soc. path. exot.*, 1918, xi, 357.

matter of conjecture, based on circumstantial evidence. It is not yet entirely clear how the leptospira happens to be carried by rats. It is probable that the infection is an accidental one, occasioned by the direct transmission of the leptospira to man through exposure of some part of the body to the water of a cesspool or to moistened ground on which the urine of an infected rat has been deposited within a few hours previously. In a longer period the organisms would be destroyed by other saprophytic bacteria.[14] The incidence of infection is much greater in countries where the body is exposed, especially the feet and hands, to ground or water infested by wild rats. The occupation of the people also plays an important part in the frequency of infection. Once a rat becomes infected it supplies the infective agent for an indefinite length of time, possibly until its death. Transmission of the leptospira from rat to rat is accomplished by infected food or drink and is particularly easy in view of their cannibalism.

The question of the presence of a leptospira in tropical countries, especially where endemic foci of yellow fever exist, has not heretofore been studied, but with the isolation of a pathogenic leptospira from certain cases of yellow fever in Guayaquil the relation between the human and animal strains demands a thorough investigation. A general survey has therefore been made to detect the presence of the leptospira among the wild animals encountered in Guayaquil.

The mode of study consisted in inoculating intraperitoneally 1 to 2 cc. of a Ringer solution emulsion of both kidneys of two animals into three guinea pigs. The emulsions were prepared, in the proportion of 1 gm. of the kidney to 10 cc. of Ringer solution, by finely grinding up the kidneys in a mortar with sterile sand. Rats, mice, bats, and an opossum were examined for the presence of the leptospira. These animals are abundant in and about the houses and buildings in Guayaquil, and they were caught and sent in alive by the Board of Health[15] of the city. Table I shows the results of the investigation.

The experiments show that eight out of twelve groups of rats carried in their kidneys a leptospira capable of producing in guinea pigs pathological changes similar to those produced by *Leptospira icteohæmorrrhagiæ*. Only two groups of rats were absolutely free

[14] Noguchi, H., *J. Exp. Med.*, 1918, xxvii, 593.

[15] I am greatly indebted to Dr. León Becerra of the Board of Health, for his cooperation in the course of the work which was carried on in Guayaquil. For some specimens of bats I am also indebted to Dr. Gonzales-Rubio, Jr.

TABLE I.

Experiments with Rats.

Groups of rats.	Guinea pig No.	Results.	Remarks.
1 (doubtful).	373	Temperature 40.2°C. on 5th day. Survived.	Perhaps an abortive infection.
	374	Temperature 39.8°C. on 5th day. Survived.	" "
	375	Apparently no reaction.	Negative.
2 (positive).	376	Temperature 40.4°C. on 6th day. Epistaxis and icterus on 7th day. Killed. Lungs and stomach hemorrhagic. Liver icteric and enlarged. Acute parenchymatous nephritis. Other organs unchanged. No leptospira found by dark-field examination.	Transfer to Guinea Pig 377 A (blood 2 cc.). Temperature 40°C. on 5th day. Killed " 8th " Typical, but no leptospira found by dark-field examination.
	376 A	Apparently no febrile reaction. Animal became icteric with temperature of 36.2°C. on 8th day. Killed. Findings typical; difficult to find leptospira in blood or organs.	In a later passage the leptospira was demonstrated.
	377	Died over night.	
3 "	378	Only slight temperature rise, but typically jaundiced on 8th day and was killed. Typical changes of the organs. Leptospira difficult to find.	
	338 A	Died in 9 days without typical changes.	
	389	Died over night.	
4 "	380	Became jaundiced on 5th day. Died in 9 days. Typical findings. Leptospira not demonstrated.	
	381	Temperature 40.2° on 10th day; 40°C. on 11th day, but animal never came down with typical symptoms.	
	382	Temperature 40.5°C. on 8th day and 37.4° on 10th day, with extreme jaundice. Typical findings with leptospira in the tissues.	

TABLE I—*Continued.*

Groups of rats.	Guinea pig No.	Results.	Remarks.
5 (negative).	383	Died of intercurrent infection in 10 days.	
	384	No reaction.	
	385	Temporary rise in temperature for several days. Survived.	
6 "	386	Died in 2 days. Negative.	
	386 A	Temperature 40.3°C. on 7th day, but with ultimate recovery.	
	387	No reaction.	
7 (doubtful).	388	Nothing typical.	
	388 A	Temperature 40.2°C. on 4th day, but recovered.	Perhaps an abortive infection.
	389	Died in 14 hours. Negative.	
8 (positive).	390	Temperature 40.2° on 5th day, 38.6°C. on 7th, with marked jaundice. Killed. Typical findings in the organs; leptospira not demonstrated by dark-field examination.	Transfer to two guinea pigs (blood 2 cc.). Both became typically yellow and were killed on 10th day. Typical findings, with leptospira in liver, but none in kidney or blood, of one animal; second animal showed no leptospira.
	390 A	Highest temperature 39°C. on 5th day. Died in 9 days. Typical lesions.	
	391	Slight febrile reaction. Recovered.	
9 "	392	Highest temperature 39.4°C. on 5th day. Died on 7th day with typical lesions.	
	392 A	Highest temperature 39.6°C. on 8th day. Died on 10th day with icterus and hemorrhages.	
	393	Temperature 40.1°C. on 6th day, 39.9° on 7th day. Transient icterus on 10th day. Recovered.	

TABLE I—*Concluded.*

Groups of rats.	Guinea pig No.	Results.	Remarks.
10 (positive).	394	No high fever. Icterus in 8 days. Killed in 11 days. Lesions typical. Leptospira difficult to find.	
	394 A	Temperature 39.4°C. on 5th day. Icterus following day. Killed on 8th day. Typical lesions at autopsy. Leptospira found.	Transfer to two guinea pigs. Both died in 7 days with typical lesions and jaundice.
	395	Remained well.	
11 "	396	Temperature 39.7°C. on 4th, 40° on 5th, 36.6° on 7th day. Intensely jaundiced. Killed in 6 days. Typical changes. Leptospira difficult to find.	Transfer to two guinea pigs (blood 2 cc. each). One died in 7 and the other in 9 days—both intensely jaundiced. Typical lesions. Melena and black vomit in latter.
	396 A	Had very little fever, but died with jaundice in 9 days. Autopsy typical.	
	397	Temperature 39.6–39.8°C. on 6th, 7th, and 8th days. Suspicion of icterus. Killed for examination in 15 days. Lungs showed old hemorrhagic areas; other organs not changed.	This animal was convalescing after a mild infection.
12 "	398	Remained well.	
	399	" "	
	400	Died in 13 days, with typical jaundice and lesions.	

from the organism, while two others showed a suspicious reaction without terminating in fatal infection. In other words, about 67 per cent of the rats studied harbored the leptospira in their kidneys.

In testing mice for the same organism it was found that out of three groups of seven mice each, one produced extreme jaundice and the other typical changes in two out of three guinea pigs, one dying in 8 and the other in 9 days after the inoculation of the kidney emulsion. There was hemorrhage before death from the rectum in one

and from the nose in the other. The leptospira was difficult to find in the blood, liver, and kidney, but was found in subsequent passages.

No positive results were obtained with the kidney emulsions of eight bats and one opossum. It may be noted that Nicolle and Lebailly[12] obtained negative results with bats caught in Tunis.

The experiments show that the emulsions which produced a fatal infection in guinea pigs did not always kill all the guinea pigs inoculated with the same quantities of the same material and under the same conditions. Some died, while others had a mild abortive infection or escaped infection altogether. This seems to indicate that there exists among individual guinea pigs a considerable variation in their susceptibility to the same strain of the organism and explains why it is important to use as many guinea pigs as convenient for the purpose of transmitting the organism to this animal. There is a close analogy with the frequent abortive infections which were obtained in the attempts to transmit *Leptospira icteroides* to guinea pigs.

The relation between the rodent strains and the strains of *Leptospira icteroides* on the one hand and those of *Leptospira icterohæmorrhagiæ* on the other was next studied from the standpoint of pathogenicity and immunity. For this purpose two strains of leptospira isolated from rats and one from mice were used. The three strains, designated Groups 8, 11, and 30, have morphological features identical with those of the strains isolated from wild rats caught in the vicinity of New York,[10] and are consequently indistinguishable from the icterohemorrhagic strains derived from the Japanese and European sources. They are slightly coarser than the strains of *Leptospira icteroides*.

With regard to the pathogenicity of the Guayaquil rat leptospira, it is difficult to point out any essential difference between the symptoms and lesions that occur in guinea pigs infected with it and those in animals inoculated with the icterohemorrhagic strains of temperate climates. They all produce jaundice and hemorrhages, although with some strains hemorrhage is the outstanding feature.

Identification of the Organism by Means of Immunity Reactions.

In order to determine whether the leptospira isolated from wild rodents in Guayaquil is identical with the strains of *Leptospira icterohæmorrhagiæ* from other sources, and what relation it may have to *Leptospira icteroides* from yellow fever patients in the same city, their immunity reactions were taken into consideration in a series of experiments.

Immune Sera.—Two rabbits were immunized with each of the three strains of Guayaquil rodent leptospira by injecting intravenously 2 to 4 cc. of rich living cultures of the organisms several times at 7 to 12 day intervals. The animals were bled on the 9th day after the last injection and the effects of their sera tested not only upon the same and other strains of Guayaquil origin, but also upon the Japanese, European, and New York strains of *Leptospira icterohæmorrhagiæ*. The relation of this group of organisms to that of *Leptospira icteroides* was likewise studied and will be discussed at greater length in connection with the relation between yellow fever and infectious jaundice.

The first experiments were designed for observation of the action of each of these sera upon the organism *in vitro*. To 0.5 cc. of a rich suspension of culture in saline solution was added 0.2 cc. of the immune serum with or without the simultaneous addition of 0.2 cc. of fresh normal guinea pig serum as complement. The mixture was placed in a water bath at 37°C. for 2 hours and then examined under the dark-field microscope. The entire procedure was carried out with strict aseptic precautions, and each experiment was accompanied by a control with normal rabbit serum. The tubes were kept at room temperature for 96 hours and their contents examined again. Except for a greater amount of precipitation in some tubes the results were about the same as those observed at the end of 2 hours at 37°C. The control tubes with normal rabbit serum showed numerous active organisms after 4 days.

Pfeiffer's phenomenon was also studied by the usual procedure; that is, examination of the peritoneal fluid of guinea pigs after inoculation with a given serum and the strain in question. In this instance 0.5 cc. of rich culture was mixed with 1 cc. of the immune serum and

injected intraperitoneally, examination being made after 30 minutes and 2 hours.

As another means of identification the protective property of each immune serum (1 cc.) was tested on guinea pigs against approximately 10 minimum lethal doses of the different strains. Unfortunately

TABLE II.

Immunological Relation of the Guayaquil Rat Leptospira to Leptospira icterohæmorrhagiæ.

Immune Serum 906, produced with Group 8 strain, injected on Jan. 15, 22, Feb. 3, 15, 1919.

Cultures tested.	Effects *in vitro*.	Pfeiffer phenomenon.	Protective property against infection in guinea pigs.
Group 8	Marked precipitation, agglutination, and disintegration.	Positive.	1 cc. protected guinea pigs against about 10 minimum lethal doses.
" 11	Effects similar to the foregoing but less in degree.	"	" "
" 30	Marked agglutination and later disintegration.	"	" "
Japanese.	Similar to the foregoing but less marked.	"	" "
American No. 1	Rather marked agglutination.	"	Could not be tested because of loss of virulence.
" " 3	Effects similar to the foregoing.	"	" "
French.	" "	"	1 cc. protected guinea pigs against about 10 minimum lethal doses.

the virulence of the American and British strains of *Leptospira icterohæmorrhagiæ* was considerably attenuated during my absence of 6 months and could not be tested with reliable results. This, however, was not a serious obstacle to determining the affinity of these strains for the Guayaquil strains, because an immune serum produced in rabbits with the avirulent American strain was tested against the pathogenic Guayaquil strains. A brief summary of the foregoing experiments is given in Tables II to VI.

TABLE III.

Immunological Relation of the Guayaquil Rat Leptospira to Leptospira icterohæmorrhagiæ.

Immune Serum 914, produced with Group 11 strain, injected on Dec. 30, 1918, Jan. 6, 14, 21, 1919.

Cultures tested.	Effects *in vitro*.	Pfeiffer phenomenon.	Protective property against infection in guinea pigs.
Group 8	Moderate agglutination but no degeneration.	Positive.	1 cc. protected guinea pigs against about 10 minimum lethal doses.
" 11	Marked precipitation and agglutination; later degeneration.	"	" "
" 30	Effects similar to the foregoing but less marked.	"	" "
Japanese.	" "	"	" "
American No. 1	" "	"	Could not be tested because of loss of virulence.
" " 3	Marked agglutination and degeneration.	"	" "
French.	" "	"	1 cc. protected guinea pigs against about 10 minimum lethal doses.

TABLE IV.

Immunological Relation of the Guayaquil Rat Leptospira to Leptospira icterohæmorrhagiæ.

Immune Serum 904, produced with Group 30 strain, injected on Dec. 30 1918, Jan. 6, 14, 22, Feb. 3, 15, 1919.

Cultures tested.	Effects *in vitro*.	Pfeiffer phenomenon.	Protective property against infection in guinea pigs.
Group 8	Marked agglutination and subsequent disintegration.	Positive.	1 cc. protected guinea pigs against about 10 minimum lethal doses.
" 11	Slight agglutination, but no degeneration.	"	" "
" 30	Marked precipitation, agglutination, lysis, and degeneration.	"	" "
Japanese.	" "	"	" "
American No. 1	" "	"	Could not be tested on account of loss of virulence.
" " 3	Slight agglutination; no degeneration.	"	" "
British.	" "	"	" "

TABLE V.

Immunological Relation of the Guayaquil Rat Leptospira to Leptospira icterohæmorrhagiæ.

Immune Serum 952, produced with American Strain 1, injected on Dec. 30, 1918, Jan. 6, 14, 22, Feb. 3, 15, 1919.

Cultures tested.	Effects *in vitro*.	Pfeiffer phenomenon.	Protective property against infection in guinea pigs.
Group 8	Moderate agglutination; slight degeneration.	Positive.	1 cc. protected guinea pigs against about 10 minimum lethal doses.
" 11	Very marked agglutination and degeneration.	"	Not tested.
" 30	Slight agglutination and degeneration.	"	1 cc. protected guinea pigs against about 10 minimum lethal doses.
Japanese.	Moderate agglutination and degeneration.	"	Not tested.
American No. 1	Marked agglutination and degeneration.	"	" "
British.	Slight agglutination and degeneration.	"	" "

TABLE VI.

Immunological Relation of the Guayaquil Rat Leptospira to Leptospira icterohæmorrhagiæ.

Immune Serum 911, produced with the Japanese strain, injected on Dec. 30, 1918, Jan. 6, 12, 22, Feb. 3, 15, 1919.

Cultures tested.	Effects *in vitro*.	Pfeiffer phenomenon.	Protective property against infection in guinea pigs.
Group 8	Moderate agglutination and degeneration.	Positive.	1 cc. protected guinea pigs against about 10 minimum lethal doses.
" 11	Slight agglutination; no degeneration.	"	" "
" 30	Marked agglutination and disintegration.	"	" "
Japanese.	Very marked agglutination and lysis.	"	" "
American No. 1	Moderate agglutination and degeneration.	"	Could not be tested because of loss of virulence.
British.	Slight agglutination and degeneration.	"	" "

The tables show that while there exist undeniable variations in the intensity of the immunity reactions as manifested *in vitro* in the form of agglutination and subsequent disintegration of the organism, as well as *in vivo* in Pfeiffer's phenomenon and protection against infection with one or the other strains, the variations are nevertheless of so slight a nature as to lead one to assume that the strains isolated

TABLE VII.

Immunological Relation of the Guayaquil Rat Leptospira to Leptospira icteroides.

Immune Serum 906, produced with Group 8 strain, injected on Jan. 15, 22, Feb. 3, 15, 1919.

Leptospira icteroides strain.	Effects *in vitro*.	Pfeiffer phenomenon.	Protective property against infection in guinea pigs.
A. A.	Slight agglutination; no degeneration.	Negative.	No protection.
E. Ch.	No effect.	"	" "
A. Ce.	" "	"	" "

TABLE VIII.

Immunological Relation of the Guayaquil Rat Leptospira to Leptospira icteroides.

Immune Serum 914, prepared with Group 11 strain, injected on Dec. 30, 1918, Jan. 6, 14, 21, 1919.

Leptospira icteroides strain.	Effects *in vitro*.	Pfeiffer phenomenon.	Protective property against infection in guinea pigs.
A. A.	No effect.	Negative.	No protection.
E. Ch.	" "	"	" "
A. Ce.	" "	"	" "
M. G.	Definite agglutination, but no immobilization.	Doubtful.	1 cc. did not prevent the infection, but the animal survived.

from rats and mice in Guayaquil belong to the group of *Leptospira icterohæmorrhagiæ* now known to be widely distributed among these rodents inhabiting the temperate zone.

The results of a parallel series of experiments performed with the immune sera on different strains of *Leptospira icteroides* are given in Tables VII to IX.

Prophylactic Inoculation.—February 6, 1919. A number of guinea pigs were inoculated subcutaneously with 0.5 and 2 cc. of killed culture (heated to 60°C. for 10 minutes in the waetr bath) of the Guayaquil rat strains of leptospira, Groups 8, 11, and 30. These animals were inoculated after 15 days (February 21) with virulent cultures of the same and of other strains. At the same time some of the vaccinated guinea pigs were inoculated also with the icterohemorrhagic strains, including the American No. 1,[16] the Japanese, and the

TABLE IX.

Immunological Relation of the Guayaquil Mouse Leptospira to Leptospira icteroides.

Immune Serum 904, produced with Group 30 strain, injected on Dec. 20, 1918, Jan. 6, 14, 22, Feb. 3, 15, 1919.

Leptospira icteroides strain.	Effects *in vitro*.	Pfeiffer phenomenon.	Protective property against infection in guinea pigs.
E. Ch.	Slight agglutination without immobilization.	Doubtful.	No protection.
A. Ce.	No effect.	Negative.	" "
M. G.	" "	"	" "
A. A.	" "	"	" "

French. The guinea pigs previously inoculated with the killed cultures of the Guayaquil strains proved resistant to a subsequent infection, not only with homologous but also with the heterologous strains derived from Japanese, European, and American sources. This experiment indicates that the Guayaquil rodent strains of leptospira are identical with *Leptospira icterohæmorrhagiæ*.

SUMMARY.

By the inoculation of guinea pigs intraperitoneally with the emulsions of kidneys from wild rats and mice captured in Guayaquil, it was found that 67 per cent of the wild rats tested harbored in their kidneys a leptospira which produced in guinea pigs symptoms and lesions identical with those produced by *Leptospira icterohæmorrhagiæ* derived either from patients suffering from infectious jaundice in Japan or Europe, or from wild rats caught in New York.

[16] This strain was so attenuated that some of the guinea pigs escaped a fatal infection and could not be used in further experiments.

Immune sera were prepared in rabbits by injecting different strains of the Guayaquil leptospira. These sera had a marked agglutinating and disintegrating influence upon the homologous strains, and also, but often to a less pronounced degree, upon the strains of *Leptospira icterohæmorrhagiæ* from other sources. Pfeiffer's phenomenon was also found to be positive, and protection was demonstrated against infection with virulent cultures of strains of *Leptospira icterohæmorrhagiæ*.

The same sera had no effect, or at most a very slight one, upon *Leptospira icteroides*. Guinea pigs inoculated with *icteroides* strains were not noticeably protected by the use of the immune sera prepared with the Guayaquil rat strains.

Guinea pigs inoculated with killed cultures of the Guayaquil strains of leptospira proved to be resistant to a subsequent infection with heterologous as well as homologous strains of *Leptospira icterohæmorrhagiæ*.

It is concluded, therefore, that the leptospira isolated from the kidneys of wild rats and mice in Guayaquil belongs to the group of *Leptospira icterohæmorrhagiæ*, and differs from *Leptospira icteroides* in its immunity reactions.

No positive transmission was obtained with kidney material from bats and an opossum.

INTESTINAL OBSTRUCTION.

II. A Study of the Factors Involved in the Production and Absorption of Toxic Materials from the Intestine.

By LESTER R. DRAGSTEDT, CARL A. DRAGSTEDT, JOHN T. McCLINTOCK, M.D., and C. S. CHASE, M.D.

(*From the Laboratories of Physiology and Pharmacology of the State University of Iowa, Iowa City.*)

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Obstruction to the passage of food through the intestine leads to adverse symptoms and complete obstruction causes death. Acute obstruction in the upper part of the small intestine is more rapidly fatal than in the intestine lower down. The symptoms are those of a severe, rapidly developing toxemia. Toxic substances accumulate in the obstructed intestine, which when injected intravenously in animals or absorbed from the abdominal cavity produce symptoms similar to those arising after acute obstruction. It has been shown by Stone, Bernheim, and Whipple (1), and later by Hartwell and his associates (2), and Murphy and Brooks (3) that the production of isolated closed loops of the intestine, with the reestablishment of intestinal continuity around the isolated loop, produces symptoms similar to those following complete obstruction of the intestine at the same level. There is an accumulation of toxic materials in these intestinal loops similar to those in the obstructed intestine. In a previous study (4) it was demonstrated that these toxic substances can be formed in such isolated closed intestinal loops, with resultant toxemia, after all food materials and digestive secretions have been previously removed by careful washing with water or salt solution. It was also shown that the secretions of the intestinal mucosa are not toxic and do not give rise to the symptoms of acute obstruction when absorbed directly from the abdominal cavity. Davis and Stone (5) have found that these secretions are not toxic even when injected intravenously in animals. This is in agreement with the observation of Murphy and Brooks. The presence of bacteria in the lumen of the intestine or in isolated closed intestinal loops is necessary for the production of the characteristic toxic substances. In the absence of bacteria, as in isolated closed intestinal loops previously rendered sterile by prolonged drainage into the abdominal cavity, many changes, even to autolysis of the mucosa through occlusion of the blood supply, may occur and no untoward symptoms result. The toxic substances can be apparently formed by the action of bacteria on the secretions of the intestinal mucosa, or on material from desquamated mucosa cells.

Davis and Stone noted that whereas normal fresh intestinal juice was not toxic, if it was kept free from preservatives and unheated, it rapidly became so, producing the same effects on intravenous injection as the materials from closed loops. A rapid and profuse growth of bacteria in this secretion was noted.

However, the presence of bacteria plus a suitable substrate either in the lumen of the obstructed intestine or in closed intestinal loops does not in many cases produce the characteristic acute toxemia unless there is some factor present permitting the absorption of these toxic materials into the general circulation. Absorption of toxic materials from the intestine occurs both in clinical and experimental obstruction and after the formation of closed isolated intestinal loops in the great majority of cases. The present study was undertaken to determine the factors involved in this absorption of toxic materials and to secure additional evidence as to the manner of their production.

The experiments were performed on dogs, under complete ether anesthesia and with strict aseptic precautions.

Isolated Closed Intestinal Loops Previously Washed with Water and Ether.

It has been demonstrated many times by different workers that the production of closed isolated loops of the small intestines in dogs, in the great majority of cases, gives rise to a quickly developing rapidly fatal toxemia in all respects similar to the symptoms of complete acute obstruction of the intestine in the same region. These closed loops in the duodenum or upper jejunum are more rapidly fatal than similar loops in the ileum or colon. In every case there accumulates in the closed loops a toxic material, similar to the material found in the intestine in experimental obstruction in animals or in acute obstruction in man. If these isolated intestinal loops are previously washed with sterile water and ether about 50 per cent of the animals survive indefinitely and display no toxic symptoms following the operation.

The mucosa of such loops when examined at varying intervals after the operation is normal in appearance. Since it has been demonstrated that the presence of bacteria in closed intestinal loops is necessary for the production of toxic materials it would appear that the ether washing of these loops previous to closure must have either

inhibited the growth of bacteria and so prevented the formation of toxic substances, or it must have changed conditions so that toxins present were not absorbed. It is conceivable that the ether may have affected conditions in any of the following ways: (1) The specific bacterium causing the elaboration of the toxic product in the closed loop may have been removed or its growth inhibited. The bactericidal property of the ether itself may have been sufficient to destroy the bacteria or the hyperemia incited by the ether as an irritant may have been instrumental in aiding phagocytosis. (2) The substrate upon which bacteria act to produce the toxic product may have been washed out by the ether. Thus the ether-soluble lecithin, a constituent of bile and animal tissues generally, is split by putrefactive intestinal bacteria giving rise to the exceedingly toxic choline and neurine. (3) The secretory activity of the mucosa may have been sufficiently depressed to prevent the accumulation of enough fluids to distend the intestine to the point of rupture or to occlude its blood supply. The recovery of the cells from the effects of the anesthetic may have been sufficiently gradual to permit the establishment of an equilibrium between the amount of secretion into the lumen of the intestine and the amount of absorption. There is a possibility that the absorptive properties of the cells of the washed loop may have been increased through the removal of the lipoid envelope of the cells by the ether. (4) The ether may have inhibited the formation of toxic substances within the cells of the mucosa of the closed loop, or produced some change in a possible harmful internal secretion of the mucosa of the intestine. (5) The ether may have decreased the absorption of substances from the intestine.

Experiments to Determine the Factors Involved in the Production of the Poisons of Obstruction.

It was found that if isolated intestinal loops were washed with water and ether previous to closure many animals survived the operation, never displayed any toxic symptoms, and that these loops when subsequently removed and examined contained great numbers of bacteria. Ether does not sterilize these intestinal loops and the efficacy of the

ether in prolonging the life of closed loop dogs does not depend primarily upon its bactericidal properties. A large number of antiseptics, such as alcohol, lysol, phenol, cresol, formaldehyde, mercuric chloride, silver nitrate, and chloroform, was used to wash the intestinal loops previous to closure. These chemicals did, in many cases, prolong the life of dogs with closed intestinal loops. Their efficacy, however, was not proportionate to their bactericidal efficiency and none was so effective as ether. In no case did they afford a complete sterilization of the intestinal loop or permanently inhibit the growth of bacteria.

The following experiments were done to determine whether the growth of bacteria and the production of the usual toxic substances in closed intestinal loops were prevented by the previous use of antiseptics. A dog which had survived the production of an isolated closed loop of the upper jejunum, previously washed with ether, was subsequently opened and the contents of the closed loop were removed with a syringe. About 50 cc. of fluid were obtained which, when injected into the abdominal cavity of another dog, caused a severe toxemia and death in 6 hours. In another animal an isolated loop of the transverse colon was made, washed with sterile water and 70 per cent alcohol, the ends were closed, and the continuity of the intestinal tract was reestablished by end to end anastomosis. This animal displayed no untoward symptoms and after 28 days was opened and the isolated loop removed. It was greatly distended, containing 225 cc. of turbid chocolate-colored fluid. The mucosa was normal. 6 cc. of this fluid were injected intravenously in another dog, and produced a profound prostration and death in 10 hours. Both the jejunal and the colon loops contained large numbers of bacteria.

These experiments established the fact that it is impossible to sterilize even a short piece of the intestinal tract by the use of chemical antiseptics. They indicate that the part played by ether is not that of a bactericide and that it does not markedly inhibit the production of toxic materials in intestinal loops. The absorption of these toxic substances is for some reason prevented. It is possible for many times the lethal dose of these poisons to remain in closed loops of the jejunum or colon without the production of toxic symptoms.

Experiments to Determine the Factors Involved in the Absorption of Poisons from the Obstructed Intestine.

The fact that closed loops of the upper jejunum in dogs are rapidly fatal, in the great majority of cases, while identical loops previously washed with ether are compatible with life in approximately 50 per cent of cases, is significant and affords an opportunity for the study of the factors involved in the absorption of the poisons of obstruction. Washing with ether does not prevent the formation of toxic materials in closed intestinal loops but it does prevent the absorption of these materials in many instances. If closed loops of the upper jejunum, untreated with ether, are made, the animal usually dies in from 1 to 5 days with symptoms of an acute toxemia, appearing only from 10 to 24 hours before death. At autopsy there is usually a perforation of the loop and a general peritonitis. Many animals, however, die and the autopsy discloses an intact loop, in most cases enormously distended, discolored, with a necrotic mucosa, but no peritonitis. It is noteworthy that these closed loop dogs display no adverse symptoms until shortly before they die at a time when apparently the accumulation of secretions in the loop has caused sufficient distention to occlude the blood supply to the mucosa. It seemed possible that ether may have prevented the onset of toxemia in closed loop dogs by preventing this distention of the loop.

Ether acts as a local narcotic causing a coagulation of the cell protoplasm. This coagulation might at least temporarily depress the secretion of intestinal juice. If this is the important factor then other substances with strong astringent but without bactericidal properties should be equally effective.

Isolated loops of the upper jejunum were made in eight dogs, flushed with water and subsequently with 8 per cent alum (aluminum potassium sulfate), the ends infolded and closed, and the continuity of the intestinal tract was reestablished by end to end suture. Two of these dogs survived the operation and remained in good health 6 months later. Six of the dogs died within the first 5 days with a perforation of the loop and a general peritonitis. Similar isolated jejunal loops were made in fourteen dogs, but a 6 per cent solution of tannic acid was used to flush the loop instead of alum. Of these dogs four con-

tinued in perfect health for months after the operation; the remainder died after periods varying from 6 to 20 days.

Apparently, simple astringents with no germicidal properties are just as effective in preventing the symptoms of toxemia in dogs with closed intestinal loops as are the antiseptics. It was evidently the astringent properties of the ether and the other chemicals rather than their bactericidal properties that account for the results produced.

The factor of distention in short closed intestinal loops is of paramount importance in the production of the toxemia, since if the distention is prevented in the majority of cases toxemia does not occur.

It has been emphasized by Hartwell and his associates and by Murphy and Brooks as well as by ourselves that toxic materials are not rapidly absorbed through the normal mucosa. This fact is well illustrated in the following experiments. Three dogs, which had survived the production of closed ether-washed loops of the upper jejunum for several weeks, were subsequently reopened. Into the closed loop of one dog the contents of a normal intestine with its bacterial flora, into the loop of the second the contents of an obstructed intestine, and into the loop of the third the evaporated ether washings of an isolated jejunal loop, containing a possible bacterial substrate, were injected. None of the animals displayed any unusual symptoms after recovering from the laparotomy and continued in good health. It has been noted before that dogs may survive the production of closed loops of the jejunum and may continue in perfect health although the loop may contain many times a lethal dose of toxic substances. This we believe is due to a protective function on the part of the intestinal mucosa which is able to inhibit or diminish the systemic effect of poisonous substances found in the alimentary tract. It is dependent upon the capacity of the mucosa cells for physiological selection by which these cells, while they absorb certain substances (in apparent defiance of physical laws) that diffuse with difficulty, do not, on the other hand, permit others to pass which may be more diffusible. This protective capacity, however, has a limit, or intoxication from the alimentary tract could not occur. Indeed it has been our experience that if long (1 to 6 feet) closed loops of the intestine, previously treated with ether, are made, no symptoms may be apparent for 2 weeks, but shortly thereafter a slowly developing toxemia may ensue and death

occur in the course of 5 or 6 days. The mucosa of these loops may be perfectly normal to both gross and microscopic examination. With short loops any poisons which may have found their way through the mucosa were apparently removed from the circulation or detoxified by the liver. With the long loops, however, the liver was not able to cope with the large quantity of toxic substances and toxemia resulted.

The following experiment emphasizes the importance of the protective action of the normal intestinal mucosa. An isolated loop (12 cm. in length) was made in the jejunum, washed with water and ether, both ends were closed, and the continuity of the alimentary tract was reestablished by end to end anastomosis around the loop. The animal quickly recovered and displayed no subsequent toxic symptoms. 1 month later a second laparotomy was done, the isolated closed loop filled with a 25 per cent solution of magnesium sulfate, dropped back, and the abdomen closed. 3 days later the animal died with the usual symptoms and autopsy revealed a perforated loop with a general peritonitis. It was demonstrated before that ether washing of the loop previous to closure does not prevent the formation of toxic materials. It is well known that a strong solution of magnesium sulfate will attract fluids into the lumen of the intestine acting through the alteration of osmotic pressure conditions. Evidently the distention of the closed loop produced by the attraction of liquids was the immediate cause of the toxemia and death. The animal was able to survive the production of a closed jejunal loop which contained more than a lethal dose of toxic materials without symptoms but died when this loop was distended. The conclusion seems inevitable that the injury to the mucosa produced by the distention has deprived these cells of their protective function and permitted an overwhelming amount of toxic material to enter the blood stream. Evidence obtained from the other workers in this field as well as from our own experiments indicates that the distention of the intestine injures the mucosa by occluding its blood supply. Once the protective layer of intestinal epithelium has been functionally destroyed absorption takes place as from the peritoneal cavity whose serosa exerts no selective action.

It is not probable that simply an irritation of the mucosa by retained substances in the obstructed intestine is the factor which damages the cells, destroys their power of selective absorption, and permits the absorption of the toxins of obstruction. There is no evidence that the toxins of obstruction are qualitatively different from the toxic materials present in the normal digestive tract and if simply an irritation of the mucosa were the important factor in their absorption a pronounced toxemia should occur in any enteritis.

Experiments to Determine the Possibility of a Non-Bacterial Origin of the Poisons of Obstruction.

In view of the fact that some of the workers, who have done a great deal of experimental work on the problem of intestinal obstruction, hold to the view that the poisons responsible for the toxemia arise independently of the intestinal bacteria, it is necessary to detail the experiments which have forced us to reject these theories. It has been postulated by a number of workers and considerable experimental and clinical evidence has been adduced to show that the toxemia incident to obstruction of the intestinal tract is due to the absorption of poisons formed in the intestinal mucosa. These poisons may be either normal products of the lining cells which, because of the obstruction, are abnormally absorbed or they may be the results of an abnormal activity on the part of the intestinal mucosa induced by the condition of obstruction. Thus Draper (6) holds that the toxemia in intestinal obstruction is due to an aberrant activity of the duodenal and probably the pancreatic cells. Whipple, Stone, and Bernheim (7) believe that death in these cases of obstruction is due to the absorption directly into the blood stream of a perverted secretion of the duodenal or upper jejunal mucosa. They assume a disturbed physiological balance of the mucosa by which abnormal products are formed and secreted into the blood stream. This toxic secretion is a proteose and, it is stated, can be formed in a mucosa in which there is no gross evidence of disturbance. Thus both of these theories exclude the intestinal bacteria as an important factor in the production of the toxic materials. A number of experiments were done to obtain evidence on this point and in no case do we believe

that the experimental evidence warrants the assumption of a non-bacterial origin of the poisons concerned in obstruction.

In our first study (4) the following points were brought out: (*a*) Open isolated loops of the duodenum, the jejunum, or the ileum do not give rise to a toxemia when their secretions are drained directly into the abdominal cavity. South and Hardt (8) had independently found that animals could survive the production of open isolated loops of the small intestine, and this has been abundantly confirmed. (*b*) Closed isolated intestinal loops previously washed with ether may produce no symptoms in 50 per cent of cases. (*c*) Closed isolated intestinal loops previously rendered sterile by prolonged drainage into the abdominal cavity produce no untoward symptoms. (*d*) Sterile closed intestinal loops produce no adverse symptoms even on complete autolysis of the mucosa through occlusion of the blood supply. (*e*) An ether-washed closed intestinal loop (not sterile), which had caused no symptoms, produced the typical toxemia and death on occlusion of its blood supply.

A consideration of these experiments indicates the improbability of a toxic secretion of the mucosa cells. It has been amply demonstrated that the normal secretions of these cells are not toxic when absorbed from the abdominal cavity or injected intravenously. The production of closed intestinal loops does not always cause toxic symptoms if the factors of bacterial growth and distention of the loops are controlled. If the toxemia were dependent upon toxic secretions of mucosa cells it should uniformly occur when closed loops are made, and furthermore one would expect a slowly developing toxemia from the time of production of the loop instead of a fulminant toxemia coincident with distention of the loop and occlusion of its blood supply. It might be conceived that distention of the intestinal loop is a necessary stimulus to the mucosa cell to produce its toxic product. But if bacteria have been previously excluded from such a closed loop it may distend to the point of rupture, or its blood supply be occluded by ligation and no toxic symptoms result. However, if bacteria are present, either of these procedures will cause typical symptoms and death. It might then be conceived that bacteria are a necessary stimulus to the production of a toxic secretion by the mucosa. However, if bacteria are present in a closed loop but the distention of the loop is prevented by substances which inhibit intestinal secretion, no toxic symptoms result. It would be too far fetched to assume both

the presence of bacteria plus a distention of the intestinal wall as a stimulus to dying cells to produce a specific toxic secretion. We know that intestinal bacteria acting upon proteins or their split products can elaborate poisons which produce symptoms identical with those of obstruction, and all the evidence we have obtained points to these substances as the important constituents of the toxins of obstruction.

It might be conceived also that obstruction and accompanying injury to the intestinal mucosa may have thrown out of function an organ essential to life. Several workers have stated, from experimental evidence, that the mucosa of the upper duodenum has a specific vital function, aside from the secretion of succus entericus and the manufacture of secretin, and that disturbance of this function rapidly causes death.

S. A. Matthews (9) reasons, from experiments in which the entire duodenum in dogs was resected, that the duodenal mucosa is as necessary for life as the adrenals or parathyroids probably through some hormone function other than that concerned with the elaboration of secretion. A. P. Matthews (10) states that if experiments are made so that the duodenal juice (succus entericus) is drained to the exterior through a fistula, the animals die with apparently the symptoms of complete extirpation of the duodenum. He suggests that death may be due to the rapid excretion of some necessary substance through the duodenum to the exterior or to the loss of some substance normally elaborated by the duodenum which is necessary to the function of the intestine lower down.

It has long been known that resection of varying lengths of the jejunum, ileum, or colon, and even a complete removal of the stomach may produce no symptoms other than can be accounted for as a result of the loss of a digestive and absorptive organ. It has also been quite generally recognized that surgical interference with the duodenum is an extremely dangerous procedure and that excision of even parts of the duodenum is usually fatal. The symptoms produced in dogs after complete extirpation of the duodenum, as described by S. A. Matthews, and those produced by drainage of duodenal juices, as described by A. P. Matthews, were so much like those produced by simple occlusion of the upper intestine, that it was considered advisable to repeat their experiments. A complete account of our work has been published in another paper (11) and accordingly only a brief summary need be given here. It was found that removal of varying lengths of the jejunum and ileum produced no other effect than some nutritional disturbance, attributable to the loss of an important digestive and absorptive organ. The mucosa of the combined jejunum and ileum does not secrete or manufacture a necessary substance and animals can survive for months after the removal of the small intestine, the duodenum and colon remaining intact. A dog was kept living 3 months after a complete removal of the pyloric

part of the stomach, the entire duodenum, and the upper jejunum. This was confirmed by Grey (12), who was able to keep a dog 9½ months after complete removal of the duodenum.

Moorhead and Landes,[1] state that they have a dog in good health and gaining weight 3 months after the removal of the entire duodenum. Thus the mucosa of the alimentary tract does not possess a function comparable with the adrenals and parathyroids. Dogs were kept living 10 and 12 days after the establishment of a fistula which drained the entire duodenum. There is no evidence that the duodenum excretes in the succus entericus any substance necessary for life, or for the function of the intestine lower down.

DISCUSSION.

It has been definitely determined that death resulting from acute obstruction of the intestine is due to a toxemia and that the responsible toxic substances are formed in the obstructed intestine. These toxic substances can be formed even if all food materials, end-products of digestion, and the secretions of the stomach, liver, and pancreas have been carefully excluded. The secretion of the intestinal mucosa is not toxic either when absorbed from the abdominal cavity or injected intravenously. The mucosa of the alimentary tract (stomach, duodenum, jejunum, ileum, or colon) does not elaborate an internal secretion which is necessary to life, or which could be disturbed by the conditions of acute obstruction so as to account for the symptom complex of that condition. The presence of bacteria in the lumen of the intestine is necessary for the production of the characteristic toxic substances and in their absence these substances do not form. They are produced by the action of the intestinal bacteria on proteins or their split products. In the absence of food, gastric juice, bile, or pancreatic juice, these bacteria can produce the characteristic toxic substances from the intestinal juice or from the proteins of desquamated mucosa cells. The important poisons will not provoke the appearance of immune bodies when injected in experimental animals (13) and we were not able to demonstrate that an animal can become immune to the toxemia of acute obstruction (14). Toxic amines are produced

[1] Moorhead, J. J., and Landes, H. E., personal communication.

by the action of various intestinal bacteria on amino-acids and the evidence more and more points to these substances as the important agents in the toxemia of acute intestinal obstruction.

The toxic substances arising in the lumen of the obstructed intestine are not readily absorbed through a normal mucosa, a point emphasized by Hartwell and his associates and by Murphy and Brooks. Nor are they absorbed to any great extent through the mucosa of a closed intestinal loop until this mucosa has been injured by the distention of the loop and the consequent interference with the blood supply. If this distention is prevented by any means absorption of poisons in quantities greater than can be cared for by the liver and other tissues does not occur. Thus it appears that the injury to the mucosa cells, either as a result of the sudden distention brought about by conditions of obstruction or by any other factors which interfere with the blood supply to the mucosa (strangulation, etc.), is an important factor in the absorption of toxic substances from the intestine. There can be no doubt that necrosis of the mucosa greatly facilitates the absorption of intestinal poisons; but it is incorrect to say that intestinal poisons, *i.e.* those found in obstruction, cannot be absorbed through a normal mucosa. The protective action of the intestinal mucosa exercised through its properties of selective absorption is not absolute, but that it is of great significance is shown by the fact that an animal can take care of the amount of poisons absorbed through the normal mucosa of a short closed intestinal loop, which has been treated with astringents, but that as soon as this mucosa becomes necrotic an overwhelming amount of toxic materials gains entrance to the blood stream, with toxemia and death occurs. The absorption in these cases cannot be different from absorption from the peritoneal cavity.

CONCLUSIONS.

1. It is impossible to sterilize the intestine by the use of chemical antiseptics even when these are applied directly to the mucosa of isolated segments.

2. The mucosa of the alimentary tract does not elaborate an internal secretion which is necessary to life, or a secretion which could be disturbed by the conditions of acute obstruction so as to account for the symptom complex of that condition.

3. The substances responsible for the toxemia in acute obstruction are produced by the action of intestinal bacteria on proteins or their split products.

4. An injury to the intestinal mucosa, particularly that resulting from disturbances of the blood supply to the intestine, greatly facilitates the absorption of these poisons. The work of Hartwell and his associates and that of Murphy and Brooks on this point are confirmed.

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A STUDY OF PNEUMOCOCCI REACTING WITH ANTIPNEUMOCOCCUS SERA OF TYPES I, II, AND III, WITH AN OBSERVATION OF A MUTATION OF ONE OF THE STRAINS.*

By MILDRED C. CLOUGH, M.D.

(*From the Medical Clinic of the Johns Hopkins Hospital, Baltimore.*)

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In the routine study of the pneumococci isolated from cases of lobar pneumonia and other infections in the wards of the Johns Hopkins Hospital from 1915–18, certain strains were encountered which were agglutinated almost equally well by antipneumococcus sera of Types I, II, and III.[1] In making these tests progressively increasing dilutions of serum were used from 1:2 to 1:64. As a rule, agglutination did not occur in such high dilutions of the sera as in the case of typical members of the fixed types in the homologous serum, nor was agglutination so prompt in appearing. No agglutination of these strains occurred in normal horse serum on isolation.

The existence of such strains has been noted by others. Mathers (1) in 1915 described such agglutination in 16 out of 31 pneumococci, classified as Group IV, isolated from patients with lobar pneumonia. This agglutination was usually absent in the higher dilutions of serum, but with two of the strains agglutination occurred with Sera I and II in a dilution of 1:128.

Wollstein and Benson (2) isolated one such strain from the sputum of a child with lobar pneumonia. The morphology of the organism was that of a pneumococcus, but inulin was not fermented, and it was "insoluble in bile and in optochin." It was, therefore, considered to be a streptococcus.

Blake (3) in a recent article states that a small number of these strains has been isolated at the Hospital of The Rockefeller Institute. He classified them as ordinary Group IV pneumococci, and considered the agglutination a non-

* This work was done in part under the Mary Putnam Jacobi Fellowship of the Women's Medical Association of New York City.

[1] These sera were obtained from the Hospital of The Rockefeller Institute, through the kindness of Dr. Rufus Cole.

specific reaction, such as may occur with other bacterial groups when undiluted serum of high agglutinin titer is used. He showed that by the use of the more delicate precipitin test a limited zone of non-specific reaction exists with pneumococci of all four types.

Stryker (4) produced strains agglutinating in antipneumococcus sera of both types (I and II) by growing Type I and II pneumococci in their homologous immune sera. Coincident with the change in agglutinability there was a decrease in virulence, with inhibition of capsule formation, and increase of phagocytability in normal serum. Absorption of Serum I or II with strains grown in homologous immune serum removed all the agglutinins for homologous and heterologous immune serum-grown strains, but not for homologous normal serum-grown strains. Saturation of serum with homologous normal serum-grown strains removed all the agglutinins for them, and also for heterologous immune serum-grown strains. After passage through a few animals these strains reverted to their original types.

Methods.

The strains reported here were morphologically typical pneumococci. Their cultural reactions will be discussed in detail later. The serological reactions of these pneumococci were studied as far as possible by the following methods.

The macroscopic agglutination of the strains was tested in progressively increasing dilutions (from 1:2 to 1:64) of Antipneumococcus Sera I, II, and III, and of normal horse serum, by adding to them an equal volume (0.25 cc.) of a 24 hour broth culture of the strain in question. These tests were incubated 1 hour in the 37°C. water bath, read, and allowed to stand in the ice chest over night, and read again the next day. The figures in the tables indicate the highest dilution of serum in which definite macroscopic agglutination was visible, without the use of a lens. Some of the strains became slightly agglutinable in normal horse serum after prolonged cultivation on blood agar, but the titer of agglutination in normal horse serum was always lower than in the antipneumococcus sera. Animal passage for a time removed this tendency to agglutination in normal horse serum.

Phagocytosis of these atypically agglutinable strains in the antipneumococcus sera was tested. Equal volumes of bacterial suspension, serum, and a suspension of washed human leucocytes were mixed in capillary pipettes, and incubated at 37°C., as in Wright's

method. Films of the mixture were then made, and stained with Hastings' polychrome methylene blue stain. The technique of preparing these suspensions, and the precautions to be observed have been described by Clough (5). In all the tests control preparations were made with normal horse serum, and the time of incubation was so adjusted as to give a maximum degree of phagocytosis in the tests with no phagocytosis in the controls. In these mixtures the presence or absence of microscopic agglutination was also noted. The degree of phagocytosis and microscopic agglutination present is recorded as (+) slight but definite; ($+^2$) marked; and ($+^3$) maximal.

Tests were then made to determine whether or not these atypically agglutinable strains elaborated a precipitable substance reacting with all three types of antipneumococcus serum, such as Dochez and Avery (6) have shown in the case of the fixed types with their homologous serum. The peritoneum of mice infected with these organisms was washed out with several cubic centimeters of salt solution, and this fluid was centrifugalized until clear. The clear supernatant fluid was then layered over sera of the various types. After noting the presence or absence of a ring at the junction of the two fluids, the tubes were shaken, and any diffuse clouding or flocculation was noted. This technique was suggested by Blake (3) as a quick method of type determination. The degree of the reactions is indicated roughly by the signs +, $+^2$, $+^3$, as in the phagocytic tests.

Agglutinin absorption tests were carried out as follows: The strains used for the absorptions were grown in 150 cc. flasks of meat infusion broth for 24 hours, and then centrifugalized at high speed until all the organisms were thrown down. The broth was pipetted off, and the organisms were resuspended in salt solution, and again centrifugalized. After removing the supernatant salt solution the organisms were killed by heating for 20 minutes in a water bath at 56°C. They were then emulsified in 5 cc. of the serum to be tested, and the mixtures incubated 2 hours in the thermostat, and left over night in the ice box. After centrifugalization the serum was tested by macroscopic agglutination tests with the strain used for the digestion to see whether absorption was complete. This process was repeated until the serum was exhausted. Usually three such absorptions were required.

The protective power of the antipneumococcus sera was tested with two strains (L and N). These organisms could be rendered sufficiently virulent by animal passage to kill mice in a dose of 0.000001 cc. In making these tests 24 hour meat infusion broth (titrated to pH 7.8) cultures were used in doses of 0.1 up to 0.000001 cc. 0.2 cc. of each culture dilution was mixed with an equal volume of the serum to be tested, and the mixture injected intraperitoneally into mice. The protective power of sera of Types I, II, and III was tested in this way, with a series of culture dilutions with normal serum as a control. With one strain the protective power of an homologous immune serum was also tested. In these tests the time of death of the inoculated mice was noted, and the presence or absence of pneumococci in the peritoneum and heart's blood was determined by films or cultures or by both. The inoculated mice were watched for 10 days. Animals dying in less than 10 days were considered to be protected if films and cultures from the heart and peritoneum showed no organisms.

With the other seven of these strains it was impossible to obtain satisfactory protection tests, because their virulence could not be increased sufficiently. With two strains (V and R) passage through ten mice, and with another strain (T) through fourteen mice in rapid succession did not result in sufficient increase in virulence to kill mice in doses of 0.1 cc. of a 24 hour broth culture.

Immune sera were prepared to seven of the nine pneumococci studied, by intravenous injection of rabbits, first with killed cultures, and later by increasing doses of broth cultures of the living organisms. These injections were continued at intervals of 2 days until the serum agglutinated the homologous organisms in a dilution of at least 1: 250. Each strain was then tested in all the sera by means of phagocytic and agglutinative reactions.

The study of these strains was attended with great technical difficulty, and not all the tests could be applied to each strain. Protection tests were possible in only two instances on account of the low virulence of the rest of the strains. Several of them required frequent animal passage in order to keep them non-agglutinable and non-phagocytable in normal serum, and three eventually became entirely avirulent for animals and had to be discarded.

Description of the Strains.

Strain 1.—V. Acute lobar pneumonia. Isolated March, 1915. *Source.*—Lung puncture. *Morphology.*—Gram-positive, lance-shaped diplococcus with small capsules. *Blood agar.*—Colonies green, small, discrete, and rather dry. *Broth.*—Diffusely clouded. *Litmus milk.*—Acidified and coagulated. *Bile.*—Partially soluble. *Inulin.*—Not fermented. *Glucose ascitic fluid agar.*—Precipitated. *Virulence.*—Low. The growth from a blood agar slant frequently failed to kill a mouse, and after ten successive animal passages 0.1 cc. of a broth culture failed to kill.

Serological Tests.

Serum.	Macroscopic agglutination.		Phagocytosis.	Microscopic agglutination.
	1 hr.	24 hrs.		
Type I	1:16	1:64	$+^3$	$+^3$
" II	1:16	1:64	$+^3$	$+^3$
" III	1:16	1:16	$+^3$	$+^3$
Normal horse	0	1:8	0	0

Strain 2.—T. · Bronchopneumonia with multiple abscesses and double empyemas. Isolated October, 1915. *Source.*—Sputum culture, and culture of lung at autopsy. *Morphology.*—Gram-positive, lance-shaped diplococcus occurring in short chains at first, and longer chains after prolonged cultivation. Small capsules. *Blood agar.*—Colonies yellowish green, rather small, discrete, fairly moist. *Broth.*—Diffuse turbidity at first, later slightly granular. *Bile.*—Partially soluble. *Inulin serum water.*—Acidified and coagulated. *Glucose ascitic fluid agar.*—Not precipitated. *Virulence.*—Low. The growth of a blood agar slant occasionally failed to kill a mouse, and after fourteen passages through mice in rapid succession 0.1 cc. of a broth culture failed to kill.

Serological Tests.

Serum.	Macroscopic agglutination.		Phagocytosis.	Microscopic agglutination.
	1 hr.	24 hrs.		
Type I	1:8	1:8	$+^2$	$+^3$
" II	1:32	1:32	$+^2$	$+^3$
" III	1:4	1:16	$+^2$	$+^3$
Normal horse	0	1:2	0	0

Agglutinin absorption tests.—Specimens of Type I and II serum were exhausted with the homologous pneumococci, and other specimens of these sera with Strain T, by the method already described. Exhaustion of both these sera with Strain T did not reduce their activity towards the homologous (Type I or II) organisms, and in general reduced but little, if at all, their activity toward the other strains in this series. On the other hand, exhaustion of Sera I and II with the homologous organism (Types I and II), reduced or eliminated their activity towards all the strains which could still be satisfactorily tested. Since by the time these sera were obtained some of the strains had become agglutinable in normal horse serum, clear-cut results could not be obtained with them.

Strain 3.—R. Bronchiectasis. Isolated May, 1915. *Source.*—Sputum (mixed culture). *Morphology.*—Gram-positive, lance-shaped diplococcus with small capsules. *Blood agar.*—Colonies resembled those of previous strain. *Broth.*—Diffuse turbidity. *Litmus milk.*—Acidified and coagulated. *Bile.*—Soluble. *Inulin serum water.*—Acidified and coagulated. *Glucose ascitic fluid agar.*—Not precipitated. *Virulence.*—Low. The growth from a blood agar slant usually killed mice, but not uniformly.

Serological Tests.

Serum.	Macroscopic agglutination. 24 hrs.	Phagocytosis.	Microscopic alggutination.
Type I	1:16	$+^{2}$	$+^{3}$
" II	1:32	$+^{2}$	$+^{3}$
" III		$+^{2}$	$+^{3}$
Normal horse	1:2	0	0

Strain 4.—X. Normal mouth. Isolated April, 1917. This culture was lost before a thorough study of it could be made. *Source.*—Mouse inoculation with saliva. *Morphology.*—Gram-positive, lance-shaped diplococcus with moderate sized capsules. *Blood agar.*—Colonies resembled those of previous strain. *Broth.*—Diffuse turbidity. *Inulin.*—Fermented.

Serological Tests.

Serum.	Macroscopic agglutination.		Phagocytosis.	Microscopic agglutination.
	1 hr.	48 hrs.		
Type I	1:4	1:32	+	$+^{2}$
" II	1:8	1:64	$+^{2}$	$+^{3}$
Normal horse	0	1:2	0	0

Strain 5.—Ba. Acute lobar pneumonia; chronic alcoholism; diabetes; diabetic coma. Isolated April, 1916. *Source.*—Sputum culture, lung puncture post mortem, and culture from heart's blood immediately post mortem,—four colonies per cc. This strain is of the greatest interest on account of certain changes in its cultural characteristics and immunological reactions which occurred while it was under observation. When first isolated this organism reacted like an ordinary atypical Type II pneumococcus. These reactions and the changes which occurred in the strain will be discussed later. After 8 months cultivation on blood agar this strain was found to be agglutinated by all three antipneumococcus sera. For convenience this form of the strain will be called Ba. *Morphology.*—Gram-positive, small, lance-shaped diplococcus with small capsules. *Blood agar.*—Colonies moderate sized, yellowish green with slight intensification of color at the edge of the colony, and a narrow zone of slight clearing around the colony. On a blood agar slant an intense green discoloration of the medium was produced around the water of condensation. *Broth.*—Diffuse turbidity. *Bile.*—Partially soluble. *Inulin serum water.*—Not fermented. *Glucose ascitic fluid agar.*—Precipitated. *Virulence.*—Low. The growth from a blood agar slant often failed to kill a mouse.

Serological Tests.

Serum.	Macroscopic agglutination. 24 hours.	Phagocytosis.	Microscopic agglutination.	Precipitin tests.
Type I	1:32	$+^3$	$+^3$	$+^2$
" II	1:64	$+^3$	$+^3$	$+^2$
" III	1:4	$+^3$	$+^3$	$+^2$
Normal horse	0	0	0	0

The second group of strains could be studied more thoroughly on account of their relatively higher virulence, and because they remained resistant to phagocytosis and agglutination in normal serum after cultivation.

Strain 6.—H. Encapsulated abscess in the region of the appendix; signs of an old peritonitis at operation. Isolated January, 1916. *Source.*—Culture of abscess at operation. *Morphology.*—Gram-positive, lance-shaped diplococcus with an occasional short chain. Moderate sized capsules. *Blood agar.*—Colonies yellowish green, discrete, fairly moist. *Broth.*—Diffuse turbidity. *Litmus milk.*—Acidified. *Bile.*—Soluble. *Inulin serum water.*—Not fermented when tested 15 months after isolation, but 4 months later, after passage through four mice, the strain had acquired or regained the ability to ferment inulin. *Glucose ascitic fluid agar.*—Not precipitated. *Virulence.*—0.1 cc. of a broth culture killed a mouse after eight successive mouse passages.

Serological Tests.

Serum.	Macroscopic agglutination.			Phagocytosis.	Microscopic agglutination.	Precipitin tests.
	1 hr.	24 hrs.	48 hrs.			
Type I	0	1:32	1:64	+	+[2]	+
" II	0	1:16	1:64	+[2]	+[2]	+[2]
" III	1:4	1:64	1:64	+[2]	+	+
Normal horse	0	0	0	0	0	0

Strain 7.—F. Bronchopneumonia following diphtheria. Isolated July, 1917. *Source.*—Culture from lung at autopsy. *Morphology.*—Gram-positive, lance-shaped diplococcus with moderate sized capsules. *Blood agar.*—Colonies green, discrete, moist. *Broth.*—Diffuse turbidity. *Bile.*—Soluble. *Inulin serum water.*—Acidified and coagulated. *Glucose ascitic fluid agar.*—Not precipitated. *Virulence.*—0.001 cc. of a broth culture killed a mouse once, but not regularly. The virulence could not be raised above this even after twelve animal passages.

Serological Tests.

Serum.	Macroscopic agglutination.			Phagocytosis.	Microscopic agglutination.	Precipitin tests.
	1 hr.	24 hrs.	48 hrs.			
Type I	1:4	1:16	1:64	+[3]	+[3]	+[2]
" II	0	1:8	1:64	+[3]	+[2]	+[2]
" III	1:8	1:32	1:64	+[2]	+	+
Normal horse	0	0	0	0	0	0

Strain 8.—L.[2] Pneumococcus meningitis. Isolated March, 1918. *Source.*—Blood culture and spinal fluid culture. *Morphology.*—Gram-positive, lance-shaped diplococcus with moderate sized capsules. *Blood agar.*—Colonies moderate sized, yellowish green with slightly lighter centers and darker edges, and a very slight zone of clearing around the colony. *Broth.*—Diffuse turbidity. *Bile.*—Soluble. *Inulin serum water.*—Acidified and coagulated. *Glucose ascitic fluid agar.*—Not precipitated. *Virulence.*—High. After cultivation for a year on blood agar and passage through two mice, 0.000001 cc. of a 24 hour broth culture still suffices to kill a mouse within 2 days.

[2] This strain was obtained through the kindness of Dr. T. P. Sprunt.

Serological Tests.

Serum.	Macroscopic agglutination.			Phagocytosis.	Microscopic agglutination.	Precipitin tests.
	2 hrs.	24 hrs.	48 hrs.			
Type I	1:4	1:64	1:64	+	+	+[2]
" II	1:4	1:16	1:64	+	+	+[2]
" III	1:8	1:16	1:16	+	+	+
Normal horse	0	0	1:2	0	0	0

Protection Tests.

Mouse No.	Dose of culture.	+Normal serum.	+Type I serum.	+Type II serum.	+Type III serum.
	cc.	*hrs.*	*hrs.*	*hrs.*	*hrs.*
1	0.1	Not done.	22	22	18
2	0.01	" "	48	26	42
3	0.001	22	S.	S.	S.
4	0.0001	24	"	"	"
5	0.00001	120	"	"	"
6	0.000001	48	"	"	"

The figures denote the number of hours between inoculation and death. Cultures or films showed pneumococci in the heart's blood and peritoneum in all cases. S. indicates that the mouse survived 10 days or longer, or died accidentally with negative cultures from the heart's blood and peritoneum. (Nos. 3 and 5 in the series protected with Type I serum.)

This test was repeated on another occasion with sera of Types I and II, and practically the same results were obtained. In this series all the mice protected with Type I serum, except Nos. 1 and 2, survived the full 10 day period, while all the control mice died with positive films and cultures.

Strain 9.—N. Acute lobar pneumonia. Isolated February, 1917. *Source.*—Sputum culture. *Morphology.*—Gram-positive, lance-shaped diplococcus with moderate sized capsules. *Blood agar.*—Colonies moderate sized, yellowish green, discrete, moist. *Broth.*—Diffuse turbidity. *Litmus milk.*—Acidified. *Bile.*—Soluble. *Inulin serum water.*—Acidified and coagulated. *Glucose ascitic fluid agar.*—No precipitation. *Virulence.*—High. 0.000001 cc. of a 24 hour broth culture sufficed to kill a mouse.

Serological Tests.

Serum.	Macroscopic agglutination.			Phagocytosis.	Microscopic agglutination.	Precipitin tests.
	1 hr.	24 hrs.	48 hrs.			
Type I	1:2	1:32	1:64	+[3]	+[3]	+[3]
" II	1:4	1:32	1:64	+[3]	+[3]	+[3]
" III	1:2	1:64	1:64	+[2]	+	+
Normal horse	0	0	0	0	0	0

Protection Tests.

Mouse No.	Dose of culture.	+Normal serum.	+Type I serum.	+Type II serum.	+N immune serum.
	cc.	*hrs.*	*hrs.*	*hrs.*	
1	0.5	Not done.	Not done.	Not done.	S.
2	0.1	" "	24	19	"
3	0.01	24	S.	S.	"
4	0.001	40	"	"	"
5	0.0001	60	"	"	"
6	0.00001	84	"	"	"
7	0.000001	36	"	"	"

Films or cultures showed pneumococci in the peritoneum and heart's blood in all cases. No. 3 with Type I serum, and No. 3 with N immune serum died accidentally with negative cultures from the peritoneum and heart's blood. This test was repeated with practically identical results.

Agglutinin absorption tests.—Type I serum was absorbed repeatedly with a typical Type I strain, and another specimen of Serum I with Strain N; and Type II serum with a typical Type II strain, and another specimen with Strain N, until completely exhausted, as described previously. These sera were then tested with typical Type I and II strains, and with the last four strains just described. The results obtained are given in Tables I and II.

These tables show that absorption of serum of Type I or II with the homologous organism removed the agglutinins for all the strains tested. On the other hand, absorption of these sera with Strain N removed the agglutinins for that strain only, and not for the homologous Type I or II strain, or for Strains H, F, and L. The removal of the agglutinins by absorption is paralleled closely by the removal of the substances stimulating phagocytosis.

TABLE I.

Macroscopic Agglutination.

Strains.	Sera.					
	Type I.	Type I, absorbed with Type I strain.	Type I, absorbed with Strain N.	Type II.	Type II, absorbed with Type II strain.	Type II, absorbed with Strain N.
Type I strain.....	1:256	0	1:256			
" II "				1:128	0	1:128
Strain N.....	1:32	0	0	1:32	0	0
" H.....	1:64	0	1:64	1:32	0	1:64
" F.....	1:64	0	1:64	1:64	0	1:64
" L.....	1:32	0	1:32	1:8	0	1:8

TABLE II.

Phagocytosis and Microscopic Agglutination.

Strains.	Sera.											
	Type I.		Type I, absorbed with Type I strain.		Type I, absorbed with Strain N.		Type II.		Type II, absorbed with Type II strain.		Type II, absorbed with Strain N.	
Type I strain.....	$+^3$	$+^3$	0	0	$+^3$	$+^3$						
" II "							$+^3$	$+^3$	0	0	$+^3$	$+^3$
Strain N.....	$+^2$	+	0	0	0	0	$+^2$	$+^3$	0	0	0	0
" H.....	+	+	0	0	+	+	+	+	0	0	+	+
" F.....	$+^2$	+	0	0	+	+	$+^2$	+	0	0	$+^2$	+
" L.....	+	+	0	0	+	+	+	+	0	0	$+^2$	+

The first figure in each column represents phagocytosis, and the second microscopic agglutination.

Summary of the Strains.

These strains may be arbitrarily divided for purposes of discussion into two groups. In the first group are five strains, L, T, R, X, and Ba. Morphologically they were typical pneumococci, with small, though definite capsules. On blood agar the colonies were green, rather small, and dry like those of Group IV pneumococci. All the strains produced a diffuse turbidity in broth and acidified milk. Two of the strains, V and Ba, resembled streptococci in that they were not completely dissolved by bile, did not ferment inulin, and caused

precipitation in glucose ascitic fluid agar. Strain T was not completely bile-soluble, but its reactions otherwise were typical of a pneumococcus. The organisms in this group, except Strain X which was not tested, were relatively avirulent, often failing to kill mice after inoculation with the entire growth of a blood agar slant.

All the organisms were agglutinated macroscopically by Antipneumococcus Sera I, II, and III. This agglutination appeared somewhat more slowly than is the case with typical members of the fixed types in their homologous serum, but the clumps of agglutinated bacteria were coarse. The exact titer of agglutination differed with the individual strains. None of the organisms was agglutinated by

TABLE III.

Macroscopic Agglutination in Antipneumococcus Sera of the Various Types.

Strains.	Sera.					
	Type I.	Type II.	Type III.	Subgroup IIa.	Subgroup IIb.	Normal horse.
V	1:64	1:64	1:16	1:8		1:8
T	1:8	1:32	1:16			1:2
R	1:16	1:32		0		1:4
X	1:32	1:64				0
Ba	1:32	1:64	1:4	0	0	0
H	1:64	1:64	1:64	0	0	0
F	1:64	1:64	1:64	0	0	0
L	1:64	1:16	1:16	0	0	0
N	1:32	1:32	1:64	0	0	0

normal horse serum on isolation, but after prolonged cultivation on artificial media slight agglutination appeared in the lower dilutions of horse serum. This tendency could be prevented for a time by frequent passage through animals. The results are given in Table III.

All the strains were phagocyted by normal leucocytes when serum of Type I, II, or III was added to the mixtures. For these organisms an incubation period of 15 minutes was found to give well marked phagocytosis in the test preparations, while the control mixtures with normal horse serum showed no phagocytosis. Longer incubations resulted in the appearance of a slight degree of spontaneous phagocytosis in the controls. Microscopic agglutination was also observed in these preparations with the antipneumococcus sera, and corrobo-

rated the results of the macroscopic tests. Table IV shows the results obtained in these tests, which were in most of the cases confirmed by several observations.

It was shown in the case of Strain Ba that a precipitable substance was elaborated in the animal body which caused the formation of a precipitate when the peritoneal washings, cleared by centrifugalization, were added to Antipneumococcus Sera I, II, and III.

Absorption of Antipneumococcus Sera I and II with Strain T removed the agglutinins for Strain T only, and not for the homologous strains, Type I or II, or for the other atypically agglutinable strains

TABLE IV.

Phagocytosis and Microscopic Agglutination in Antipneumococcus Sera of the Various Types.

Strains.	Sera.											
	Type I.		Type II.		Type III.		Subgroup IIa.		Subgroup IIb.		Normal horse.	
V	$+^3$	$+^3$	$+^3$	$+^3$	$+^3$	$+^3$	0	0	0	0	0	0
T	$+^2$	$+^3$	$+^3$	$+^2$	$+^2$	$+^3$	0	0	0	0	0	0
R	$+^2$	$+^3$	$+^2$	$+^3$	$+^2$	$+^3$	0	0	0	0	0	0
X	+	$+^2$	$+^2$	$+^3$	0	0	0	0	0	0	0	0
Ba	$+^3$	$+^3$	$+^3$	$+^3$	$+^3$	$+^3$	0	0	0	0	0	0
H	+	$+^2$	$+^2$	$+^2$	$+^2$	+	0	0	0	0	0	0
F	$+^3$	$+^3$	$+^3$	$+^2$	$+^2$	+	0	0	0	0	0	0
L	+	+	+	+	$+^2$	+	0	0	0	0	0	0
N	$+^3$	$+^3$	$+^3$	$+^3$	$+^2$	+	0	0	0	0	0	0

The first figure in each column denotes phagocytosis, and the second microscopic agglutination.

reported here. On the contrary, exhaustion of these sera with the homologous organisms removed, also, all the agglutinins for Strain T and the other strains studied.

In the second group are Strains H, F, L, and N. Morphologically they were typical pneumococci with well developed capsules. The colonies on blood agar were yellowish green, discrete, moderate sized, and fairly moist, resembling more the colonies of pneumococci of Type I or II. All these organisms produced a diffuse turbidity in broth and acidified litmus milk. All the strains were dissolved by bile, and all fermented inulin. None of the strains caused any pre-

cipitation of glucose ascitic fluid agar. In general the virulence of these organisms was greater than that of the preceding group. Strain H killed mice once with a dose of 0.1 cc., and Strain F once with a dose of 0.001 cc. of a 24 hour broth culture. Strains L and N, however, were highly virulent, and killed mice repeatedly in a dose of 0.000001 cc.

All these strains were agglutinated macroscopically by Antipneumococcus Sera I, II, and III, even in a dilution of 1:64. This agglutination took place more slowly than in the preceding group, and the clumps were somewhat smaller. No agglutination occurred in normal horse serum even after long cultivation on blood agar. The results of these tests are given in detail in Table III.

All the strains were phagocyted in the presence of the antipneumococcus sera. Long incubations, from 1 to 2 hours, gave the best results with these strains. No phagocytosis was ever observed in the normal horse serum controls. Microscopic agglutination also occurred in the mixtures, and confirmed the results obtained by the macroscopic method. Table IV shows the results of these tests.

It was shown that all these strains elaborated a soluble substance in the animal body which caused the formation of a precipitate on the addition of serum of Type I, II, or III. No precipitate resulted when normal horse serum was added.

Antipneumococcus Sera I and II were absorbed with the homologous strain, and also with Strain N. The sera exhausted with the homologous strains showed no agglutinative or phagocytic activity towards the homologous strains, or towards any of the strains in this study. On the other hand, the sera (Types I and II) exhausted with Strain N were as active on typical Type I and II organisms, and on all the atypically agglutinable strains with which they were tested, except Strain N, as were the unabsorbed sera. The agglutinins concerned in the reaction are, therefore, to be regarded as minor agglutinins. The behavior of the bacteriotropins was similar to that of the agglutinins.

Strains N and L were of sufficient virulence to permit testing the protective power of the antipneumococcus sera. Both strains killed mice in all doses used down to 0.000001 cc. when normal serum was added to the culture dilutions. With Strain L, Sera I, II, and III

protected all the animals receiving 0.001 cc. or less (1,000 times the minimal lethal dose). With Strain N, Sera I and II protected all the mice receiving 0.01 cc. or less (10,000 times the minimal lethal dose). The homologous immune serum (Strain N) was found to protect mice from 0.5 cc. of the culture (500,000 times the minimal lethal dose).

The results demonstrate that a serum may possess protective power against an organism for which it possesses only minor agglutinins, and are in accord with the findings of Avery (7) in regard to the behavior of atypical Type II organisms with Type II serum.

This protective power for mice suggests the possibility that Type I (or II) serum might have some therapeutic value in cases of human infection with these organisms.

In order to determine whether or not there existed interrelationships between these pneumococci which reacted with Antipneumococcus Sera I, II, and III, an immune serum was prepared to seven of the nine strains studied. The serum having the highest titer was that produced against Strain T. This serum agglutinated the homologous organism in a dilution of 1:2,000.

As far as possible each strain was tested in all the immune sera. The results of phagocytic tests and the presence or absence of microscopic agglutination are shown in Table V, and the results of macroscopic agglutination tests in Table VI. In general, all the strains were serologically distinct, though there seemed to be some interrelationships between certain strains. Strain V was agglutinated and phagocyted actively in the serum of Strain R, as well as in the homologous serum; and Strain R, similarly, in serum of Strain V. Strains H, F, and N also showed some degree of cross-agglutination and phagocytosis, though with these organisms agglutination and phagocytosis were less active in the heterologous sera than in the homologous serum. The interreactions of Strains H and N were demonstrated also by precipitin tests, and a slight precipitin reaction was also observed with Strain F when tested with Serum H.

These sera caused no agglutination or phagocytosis of typical members of Type I or II, or of Subgroups IIa, IIb, or several other atypical Type II strains which were tested.

A possible relation between the pneumococci agglutinating with Antipneumococcus Sera I, II, and III and the fixed types is suggested by certain changes which occurred with Strain B while it was under observation.

This organism was isolated April, 1916, from a case of diabetes with a terminal lobar pneumonia, by sputum culture, lung puncture, and blood culture. On isolation it was agglutinated macroscopically by Serum II in a dilution of 1:8, while no agglutination occurred with Serum I. No phagocytosis occurred with Serum I or II, though microscopic agglutination was observed in the preparation with Serum II. As shown by Clough (5), this reaction is characteristic of atypical

TABLE V.

Crossed Phagocytic and Agglutinative Reactions.

Strains.	Immune sera.														Normal rabbit serum.	
	V		R		T		Ba		H		F		N			
V	$+^3$	$+^3$	$+^3$	$+^3$	0	0	0	0	0	0			0	0	0	0
R	$+^2$	$+^2$	$+^3$	$+^3$	0	0	0	0	0	0			0	0	0	0
T	0	0	0	0	$+^3$	$+^3$	0	0	0	0			0	0	0	0
X	0	0			0	0									0	0
Ba	0	0	0	0	0	0	$+^3$	$+^3$	0	0	0	0	0	0	0	0
H	0	0	0	0	0	0	0	0	$+^3$	$+^3$	+	0	+	+	0	0
F	0	0	0	0	0	0	0	0	$+^2$	+	$+^3$	+	$+^2$	+	0	0
N	0	0	0	0	0	0	0	0	+	0	$+^2$	+	$+^3$	$+^3$	0	0
L	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

The first figure in each column denotes phagocytosis, and the second microscopic agglutination.

Type II pneumococci. The organism was considered, therefore, to be an atypical Type II.

This strain was cultivated on blood agar (with three intervening animal passages) for 8 months, and was then tested again. At this time the strain had become agglutinable and phagocytable in Antipneumococcus Sera I, II, and III. The exact cultural and serological reactions obtained have been described previously (Strain 5). This strain will be referred to as Ba.

Between January and March, 1917, this organism was passed through seven mice. When tested at this time it was still agglutinated macroscopically with Sera I, II, and III and not with normal horse serum. After passage through two more mice it was again tested as to its phagocytability in these sera. No phagocytosis or microscopic agglutination occurred with sera of Type I or III,

or with normal serum. With Serum II no phagocytosis was observed, but there was well marked microscopic agglutination. Tests were then made with a Subgroup IIa serum[3] obtained from the Hospital of The Rockefeller Institute, with two specimens of Subgroup IIa serum prepared in this laboratory, and also with the active postcritical sera of two patients with Subgroup IIa infection. Marked phagocytosis and agglutination occurred with all these sera.

A serum was then prepared by immunizing a rabbit against this strain, which will be referred to for convenience as Bb. This Bb serum caused marked phagocytosis and agglutination of the homologous strain, and also of three other Subgroup IIa strains with which it was tested. It caused no phagocytosis or microscopic agglutination of a typical Type I or II strain. Macroscopic agglutinations corroborated the results of microscopic agglutinations. This serum caused no phagocytosis or agglutination of Strain Ba. It seemed clear, then, that Strain

TABLE VI.

Crossed Macroscopic Agglutination Reactions.

Strains.	Immune sera.							Normal rabbit serum.
	V	R	T	Ba	H	F	N	
V	1:256+		0					0
R	1:64	1:256	0		0			0
T	0	0	1:2,048		0			0
X	0		0					0
Ba	0	0	0	1:256+	0	0	0	0
H	0	0	0	0	1:512	0	1:32	0
F	0	0	0	0	1:16	1:64	0	0
N	0	0	0	0	0	0	1:512	0
L	0	0	0	0	0	0	0	0

Bb was a typical Subgroup IIa strain, and differed serologically from Strain Ba. If errors could be excluded, it seemed probable, therefore, that a mutation had occurred.

In order to confirm this finding, and to exclude the possibility of a confusion of strains, Strain Ba was then passed through another series of mice. The strains in this series will be referred to as Ba followed by a figure which indicates the number of mouse passages. After each passage the strain was recovered by plating the heart's blood on blood agar, and subculturing from a single colony to eliminate as far as is possible in this way the chance that we were dealing with a mixture of strains, and so satisfy the pure line requirement.

[3] This serum was obtained from the Hospital of The Rockefeller Institute, through the kindness of Dr. Alan M. Chesney.

Serum was obtained by immunizing a rabbit to a strain of this Ba series. This serum caused marked phagocytosis and agglutination of the first Ba strain, and also of the succeeding Ba strains, but was without any effect on the Bb strains.

No change occurred in the strains of this series during the first five animal passages. They were agglutinated macroscopically by the Ba serum, but not by a Subgroup IIa serum. After the sixth mouse passage, however, Strain Ba6 failed to become agglutinated macroscopically by Ba serum, but was agglutinated by a Subgroup IIa serum. This change was corroborated by phagocytic tests, the results of which are tabulated below.

Serological reactions of Strain Ba6.	Phagocytosis.	Microscopic agglutination.
Strain Ba6 + Type I serum	0	0
" Ba6 + " II "	0	$+^3$
" Ba6 + Ba serum	0	0
" Ba6 + Bb "	$+^3$	$+^3$
" Ba6 + Subgroup IIa serum	$+^3$	$+^3$
" Ba6 + " IIb "	0	0
" Ba6 + normal horse and rabbit serum	0	0

It seemed probable, then, that the same definite changes in serological reactions previously observed must have again occurred during the sixth mouse passage.

Strain Ba5 was then passed through another mouse to see whether this strain would again change into a Subgroup IIa. The strain then obtained was labelled Ba6a. The reactions of this strain follow.

Serological reactions of Strain Ba6a.	Macroscopic agglutination.	
	2 hrs.	24 hrs.
Strain Ba6a + Ba serum	1:64+	1:64+
" Ba6a + Bb "	1:32	1:32
" Ba6a + normal horse serum	0	0

Serological reactions of Strain Ba6a.	Phagocytosis.	Microscopic agglutination.
Strain Ba6a + Type I serum	+	$+^3$
" Ba6a + " II "	$+^2$	$+^3$
" Ba6a + " III "	$+^2$	$+^2$
" Ba6a + Subgroup IIa serum	$+^3$	$+^3$
" Ba6a + " IIb "	0	0
" Ba6a + Ba serum	$+^2$	$+^2$
" Ba6a + Bb "	$+^2$	$+^2$
" Ba6a + normal horse serum	0	0

From these results it seemed likely that Strain Ba6a was a mixture of the two forms, one corresponding to the Ba strains agglutinating with all three antipneumococcus sera, and one corresponding to the Bb (Subgroup IIa) strains. Indeed, in the preparations of Strain Ba6a there was some difference in the morphological appearance of the organisms, those which were agglutinated by the Bb and Subgroup IIa sera being larger, more loosely clumped, and having moderate sized capsules; while those agglutinated by the Ba serum appeared smaller, and the clumps were denser. With the specimen of stain used for these preparations the capsules on the latter organisms were not stained.

This culture Ba6a was plated out, and two types of colonies were fished, one of which grew with a delicate growth on blood agar (called Ba6a-Subgroup IIa form), while the other grew more luxuriantly, and stained the base of a blood agar slant an intense green (called Ba6a-undifferentiated form). These two strains were then tested for phagocytosis and microscopic agglutination in Ba and Bb sera and in Antipneumococcus Sera I and II. The results of the tests follow.

Serological reactions of both forms of Strain Ba6a.	Phagocytosis.	Microscopic agglutination.
Strain Ba6a (undifferentiated form) + Type I serum	+	+[2]
" Ba6a " " + " II "	+	+[3]
" Ba6a " " + Ba serum	+[3]	+[3]
" Ba6a " " + Bb "	0	0
" Ba6a " " + normal horse serum	0	0
" Ba6a (IIa form) + Type I serum	0	0
" Ba6a (IIa ") + " II "	0	+[3]
" Ba6a (IIa ") + Ba serum	0	0
" Ba6a (IIa ") + Bb "	+[3]	+[3]
" Ba6a (IIa ") + normal horse serum	0	0

The results of the tests with Sera I and II were confirmed by macroscopic agglutinations.

Serological reactions of both forms of Strain Ba6a.	Macroscopic agglutination.	
	1 hr.	24 hrs.
Strain Ba6a (undifferentiated form) + Type I serum	1:8	1:16
" Ba6a " " + " II "	1:64	1:64
" Ba6a " " + normal horse serum	0	0
" Ba6a (IIa form) + Type I serum	0	0
" Ba6a (IIa ") + " II "	0	1:16
" Ba6a (IIa ") + normal horse serum	0	0

Furthermore the reaction of these strains with inulin serum water differed, Strain Ba6a (IIa form) fermenting it with the production of acid and coagulation, while Strain Ba6a (undifferentiated form) did not ferment it.

To summarize the changes, on artificial cultivation an atypical Type II pneumococcus (B original) changed in its serological reactions into one of the atypically agglutinable organisms (called Ba) reacting with Antipneumococcus Sera I, II, and III. That this change was not due to a confusion of strains was proved by testing Ba serum with all the other strains in the laboratory reacting in this way, and also by testing the Ba organism with the immune sera produced against all the atypically agglutinable strains. No interreactions occurred.

After animal passage Strain Ba changed back abruptly into a strain, Bb, having the phagocytic and agglutinative reactions of a Subgroup IIa. These two strains, Ba and Bb, were serologically distinct, and immune sera produced against each had no effect on the other strain.

In order to confirm this finding, Strain Ba was again passed through a series of mice, and again changed abruptly into a Subgroup IIa form. From the last culture in this series reacting with the Ba and not with the Bb serum, a third series of mice was started in an attempt to produce again a mutation. A change occurred during the next animal passage, and the strain recovered from this mouse was shown to be a mixture of the a and b forms. These forms were separated by plating. The a form reacted with the Ba serum and also with Antipneumococcus Sera I and II, but not with the Bb serum; while the b form reacted with the Bb serum, and with Subgroup IIa serum, but not with the Ba serum. It was not acted on by Type I serum, and was agglutinated but not phagocyted in Type II serum. The morphological and cultural reactions of these strains also differed. The a form had small capsules, grew profusely, and colored diffusely the base of a blood agar slant an intense green, did not ferment inulin, and was only partially soluble in bile. The b form had well developed capsules, grew delicately with no diffuse staining of the blood agar, fermented inulin, and was completely soluble in bile. It seemed definite, then, that a mutation of this organism had occurred.

Mutations of various species of bacteria have been noted by a number of observers. Changes in the morphological and cultural characteristics of streptococci and pneumococci which were regarded as mutations of one species into the other have been observed by Buerger and Ryttenberg (8), Schereschewsky (9), Davis (10), Aschner (11), and others.

Rosenow (12) has reported not only cultural and morphological changes indicating mutations between *Streptococcus mucosus*, pneumococcus, *Streptococcus viridans*, *Streptococcus rheumaticus*, and *Streptococcus hæmolyticus*, but also mutations of one type of pneumococcus into another based on serological reactions. A typical Type I pneumococcus was converted by animal passage into a Type II pneumococcus, and again into a hemolytic streptococcus. Likewise streptococci were transformed into Type I and II pneumococci. A Pneumococcus Type I sent from The Rockefeller Institute was agglutinated by Serum I when grown in all media except ascites dextrose agar, but when grown in this medium was agglutinated only by Serum II. A Pneumococcus Type II from the same source was agglutinated specifically when grown on ascites dextrose agar, but was agglutinated by both Sera I and II when grown on other media.

Such mutations from one type of pneumococcus to another have not been confirmed by others. Cole (13) states that in the study of several hundred strains of pneumococci, during which some have been under artificial cultivation for 3 years, and some have been passed through as many as 100 animals, no transformations have been noted.

Our experience also indicates that, while slight variations in cultural characteristics such as bile solubility and inulin fermentation are not unusual, such mutations as Rosenow described are not of frequent occurrence. Several Type I and atypical Type II strains, which have been under artificial cultivation for 4 years, one Type II strain for 3 years, and several Type III strains for 2 years have shown no changes in their cultural or serological reactions. It is possible, however, that pneumococci which are less strictly parasitic, and possibly less highly differentiated, may be more susceptible to mutation than are the fixed types.

Although no further proof has been obtained on this point, this mutation of Strain B suggests that these pneumococci reacting with antipneumococcus sera of all three types may represent primitive, relatively undifferentiated forms of pneumococci from which the fixed types may have arisen.

SUMMARY.

In this paper are reported the results of a study of nine strains of pneumococci agglutinating with antipneumococcus sera of all three types (Nos. I, II, and III). Seven of the strains were the cause of serious or fatal infections in human beings.

Morphologically they were typical pneumococci with characteristic growth on ordinary media. Most of the strains were soluble in bile, fermented inulin, and caused no precipitation on glucose ascitic fluid agar. Two of the strains, however, resembled streptococci in these three cultural characteristics, but have been regarded as pneumococci on account of their serological reactions. Variations in the cultural reactions occurred with several strains while they were under observation.

The virulence of the strains varied greatly, some strains being almost non-pathogenic, and others killing mice in doses of 0.000001 cc. of a 24 hour broth culture.

Antipneumococcus Sera I, II, and III agglutinated all the strains in fairly high dilution (1:8 to 1:64 or higher), while normal horse serum caused no agglutination.

Antipneumococcus Sera I, II, and III stimulated active phagocytosis of all the strains, while no phagocytosis occurred in control preparations with normal horse serum.

These strains elaborated a soluble substance in the body of inoculated mice which caused the formation of a precipitate when the peritoneal washings, cleared by centrifugalization, were added to the antipneumococcus sera of all three types.

Antipneumococcus Sera I, II, and III protected mice equally well against 1,000 to 10,000 times the minimal lethal dose of the two strains with which protection tests could be carried out.

Absorption of serum of Types I and II with the homologous pneumococcus removed the agglutinins and the bacteriotropins for all

these strains. Absorption of these sera with Strains T and N removed the agglutinins and the bacteriotropins for the homologous strain only, and not for typical members of Type I or II, or for the other atypically agglutinable strains reported in this paper. The agglutinins concerned in the agglutination of these peculiar strains are therefore minor agglutinins.

As shown not only by agglutination tests, but also by protection tests and agglutinin absorption tests, these organisms bear the same relation to Types I, II, and III, as do atypical Type II strains to Type II.

Immune sera were prepared with these strains, and each strain was tested with all the immune sera by means of phagocytic and agglutinative reactions. In general, the strains were found to be serologically distinct, though some interrelationships existed between Strains V and R, and between Strains H, F, and N. These sera had no activity towards strains belonging to Type I or II, or atypical Type II.

A mutation occurred in one of the strains, B, while it was under observation. On isolation this strain had the cultural reactions of a typical pneumococcus, and had the phagocytic and agglutinative reactions of an atypical Type II. After 6 months cultivation on blood agar its serological reactions changed, and it became actively phagocyted and agglutinated in antipneumococcus sera of Types I, II, and III. Its cultural characteristics also changed, and it became bile-insoluble, did not ferment inulin, and caused precipitation in glucose ascitic fluid agar. At this time it caused an intense green discoloration at the base of the blood agar slants around the water of condensation. By repeated animal passages this strain was three times made to revert abruptly to its original form (atypical Type IIa), both in cultural and serological reactions. An immune serum was prepared to each form of the strain, and each serum acted strongly on the homologous form, but was without action on the heterologous form of the strain.

This mutation suggests that these pneumococci reacting with all three types of antipneumococcus sera may represent primitive, relatively undifferentiated forms from which the fixed types may have arisen.

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STUDIES OF OXYGEN IN THE VENOUS BLOOD.

V. Determinations on Patients with Anemia.

By CHRISTEN LUNDSGAARD, M.D.

(*From the Medical Clinic of the University of Copenhagen, Copenhagen, Denmark.*)

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In the first four articles of this series (1–4) facts were discussed which indicate that the oxygen content of the venous blood depends on a number of factors: (1) the total oxygen-combining power, or the hemoglobin content of the blood; (2) the degree of oxygen saturation of the blood in the lungs; (3) variations in the metabolism of the tissues drained by the vein tapped, as compared with the metabolism of the rest of the body; (4) variations in the rate of blood flow through the tissues drained compared with the rest of the body; (5) variations in the minute volume of the heart.

In order to find the effect of one of these factors on the oxygen content of the venous blood, it is necessary to control the other factors or keep them constant.

In Papers II, III, and IV we studied in cardiac patients the effect of the fifth of the above factors. It was not possible to exclude completely the effects of the other four factors, but by choosing suitable subjects, *viz.* decompensated cardiac patients and normal controls, and by excluding the other factors as nearly as possible, or by correcting for them, we were able to observe with a certain degree of accuracy the effect of the cardiac output on the venous oxygen. In particular, variations in the hemoglobin content were corrected for by basing our conclusions not on the absolute value of the venous oxygen, but on the oxygen unsaturation, or the difference between the venous oxygen and the total oxygen capacity of the blood. The correctness of following this procedure in individuals with varying hemoglobin content was assumed rather than exactly proved.

This paper presents the proof in a study of the venous oxygen content and unsaturation in a series of patients in whom the hemoglobin varied over a wide range.

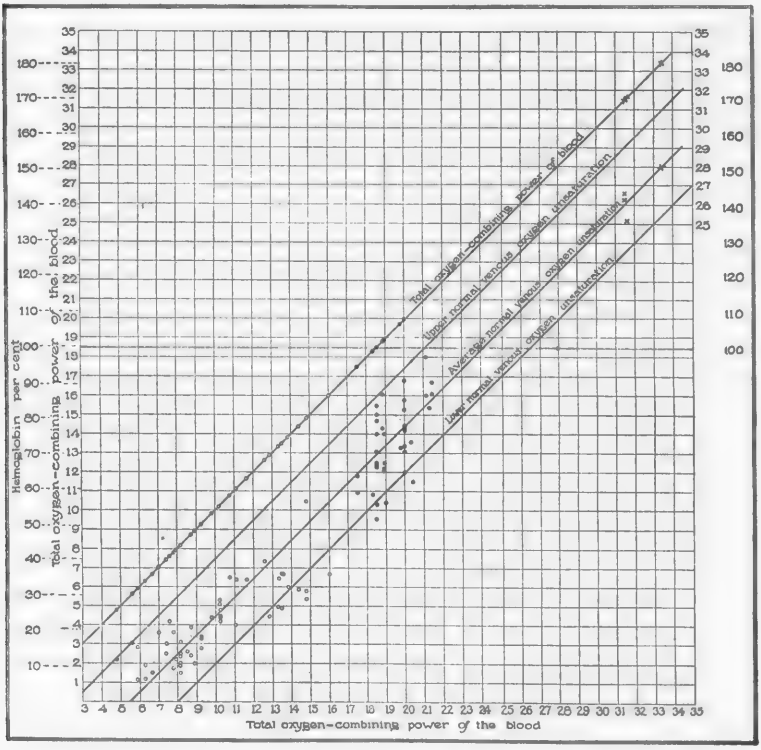
Nine patients with anemia and one patient with polycythemia[1] were selected for this purpose. The hemoglobin in these cases varied from 181 to 27 per cent. The oxygen-combining power of the blood varied consequently from 33.40 to 5.00 volumes per cent. The lungs were examined before each determination and did not show any pathological changes, which might influence the saturation of the blood. The blood samples were drawn at least 2 hours after a meal and after 10 minutes rest in bed. Nothing was found to indicate any abnormality in the circulation, apart from a slight increase in the pulse rate in some of the patients. The diuresis and the blood pressure were found to be normal.

The technique in drawing the blood samples was exactly the same as described in Paper I of this series. In determining the oxygen of the venous blood Van Slyke's method was used (5). The total oxygen-combining power of the blood was either determined directly—Van Slyke's method—or calculated from the hemoglobin determined by Haldane's method.

The results on the patients with anemia are given in Tables I to X. As a whole, the values of the oxygen in the venous blood increase proportionally with the increase in the hemoglobin, giving values for the oxygen unsaturation, which vary within the ordinary normal limits—2.5 and 8 volumes per cent. In a few instances the unsaturation has been between 8 and 10 volumes per cent. This was found only in patients in which hemoglobin had increased rather quickly from low values to about the normal. In other words, the amount of oxygen taken away from the blood during passage through the body capillaries in resting individuals is practically independent of the hemoglobin content of the blood. In order to make the relations more clear the results are presented in Text-fig. 1, where the abscissæ and the ordinates indicate oxygen-combining power in volumes per cent and hemoglobin in per cent. The corresponding percentages are also indicated, as calculated on Haldane's average, 18.5 per cent oxygen equalling 100 per cent hemoglobin. On the upper oblique line are the values for the total oxygen-combining power in each given instance

[1] The details on this patient will be published in the next number of this *Journal*, but the values for unsaturation are given in Text-fig. 1 of this paper.

and on the ordinate belonging to this point is indicated the value for the oxygen of the venous blood; the distance from this figure to the base-line gives the venous oxygen content, while the distance from the point to the upper oblique line measures the venous unsaturation.


Hemoglobin per cent
Total oxygen-combining power of the blood
Total oxygen-combining power of blood
Upper normal venous oxygen unsaturation
Average normal venous oxygen unsaturation
Lower normal venous oxygen unsaturation
Total oxygen-combining power of the blood

TEXT-FIG. 1. Values for the total oxygen-combining power and the venous oxygen in individuals with different amounts of hemoglobin. The circles with light centers indicate values for anemic patients, the solid black circles values for normal individuals, and the crosses values for a polycythemic patient.

The values of the oxygen-combining power lie, of course, on a straight line which goes through the zero point at an angle of 45° with the base-line. If the oxygen unsaturation were absolutely constant the values for the oxygen of the venous blood would also lie on a

straight line, which would be parallel to and below the upper one. This is, however, not to be expected. Even in normal individuals, as previously shown, rather extensive variations occur. On the diagram the results reported in the present paper are combined with those obtained in a series of normal individuals and published in Paper I. The earlier figures cover the middle area between the anemic patients and the polycythemic patient reported below. The diagram shows that it was justifiable to use the oxygen unsaturation in order to exclude the influence of variations in the hemoglobin content of the blood. The variations in the oxygen unsaturation in these cases are probably due to small variations in one or several of the other previously named factors. Of these, oxygenation in the lungs is probably the most constant (in these patients), the minute volume and the local blood flow probably the most variable.

These results are of interest not only for the interpretation of oxygen determinations in general, but also for the understanding of the circulatory mechanism in patients with anemia.

The fact that the hemoglobin is the only bearer of oxygen from the lungs to the tissues has made it a question of moment in what way anemic patients are able to compensate for the decrease in oxygen-combining power of the blood. For a considerable period of time it was supposed that no compensation was necessary, for the reason that the total metabolism was considered to be lower in anemic patients. This theory was based on experiments on dogs by Bauer (6) and on conclusions from fatty degenerations in the organs of patients dying from anemia, the fat being considered the result of lack of oxygen. Experiments by Kraus (7) and others showed that the metabolism is not diminished in patients with anemia. This being the case, a compensatory mechanism for the lack of hemoglobin was thought necessary.

Three theories were put forward: (*a*) an increased oxygen capacity of the hemoglobin—increased "specific capacity" (Hoppe-Seyler (8), Bohr (9), and his pupils), (*b*) an increase in the blood flow, (*c*) an increased percentile consumption of the oxygen in the blood. After Butterfield (10), Barcroft and Morawitz (11), Masing and Siebeck (12), and von Reinbold (13), who proved the proportionality between the color index and the oxygen-combining power of the hemoglobin, the theory about the "specific oxygen capacity" is untenable.

From the experimental data in the literature it is impossible to decide between the other two theories. In some experiments the blood flow has been found increased (Kraus (14) and Plesch (15)). Other experiments are interpreted as showing increased consumption; for instance, experiments of Finkler (16). Morawitz and Roehmer, who have determined the oxygen in the venous blood in eighteen patients with anemia and one with polycythemia, interpret their results to indicate that both methods of compensation are used by the anemic organism.

In discussing the problem it is evidently necessary to distinguish between the resting and working organism. In a resting organism the rate of oxygen consumption is approximately a constant for each particular individual, and for different individuals within the same species it is constant except for variations caused by differences in the surface and weight of the body. The increase caused by exercise is, of course, dependent on the extent of the work. From the determination of the minute volume of the heart and the oxygen unsaturation of the venous blood, we know that the normal resting organism on the average uses 5.5 volumes per cent oxygen in the capillaries. The normal oxygen capacity of the blood being 18.5 volumes per cent, the normal individual consequently has 13 volumes per cent of unused oxygen in his veins. This amount of oxygen may be termed the reserve oxygen, and looked upon as analogous to the reserve force of the heart. When the organism for one reason or another needs more oxygen, or when the blood flow in the capillaries is slower than normally, the organism uses the reserve oxygen. Anemia is in this respect simply a condition where the amount of reserve of the blood is diminished in the same degree as the hemoglobin; the opposite takes place in polycythemia. From a purely mechanical point of view it is clear that in a resting individual no compensation is needed until the hemoglobin has fallen so low that all the reserve oxygen is used in each circulation. This occurs when the oxygen-combining power of the blood has fallen to 5.5 volumes per cent, which corresponds to 30 per cent hemoglobin. Above this value no compensation seems necessary; below this the organism must compensate for the lack of reserve oxygen storage in the blood, and this can be done only in one way, by an increased output of the heart.

The results of the determination in this paper show that this viewpoint is as a whole correct. The last residuum of the oxygen in the blood—the last part of the reserve oxygen—is taken away by the body cells in resting individuals just as easily as the first part. This, of course, is of great importance for the body, especially for the heart.

Clinical experience agrees with the experimental results in this paper; a bodily and mentally resting anemic patient usually does not show any marked reaction from the circulation, until the hemoglobin percentage has fallen below about 30. In patients whose hemoglobin is below that value we usually find an increased pulse rate and palpitation of the heart even at rest.

SUMMARY.

1. Determinations of the oxygen content and the oxygen unsaturation of the venous blood have been performed on patients with varying amounts of hemoglobin.

2. The oxygen unsaturation of the venous blood is independent of the oxygen capacity, unless the latter is reduced below the normal value for oxygen unsaturation (about 5 volumes per cent). In a polycythemic patient,[1] for example, with 33.4 volumes per cent oxygen capacity (181 per cent hemoglobin), the venous oxygen content was 28 volumes per cent, giving an unsaturation of 5.4 volumes per cent. Similarly, in an anemic patient with only 6.7 volumes per cent oxygen capacity (36 per cent hemoglobin), the venous oxygen was 1.5, giving an unsaturation of 5.2 volumes per cent. This means that the tissues extract from the blood all the oxygen they need with apparently equal readiness, regardless of whether the extraction leaves a great oxygen reserve in the blood as in polycythemia, or practically no reserve as in anemia.

3. The results seem to show that the resting organism does not increase its circulation until all the reserve oxygen is used. This means that the resting anemic organism does not need or use any compensation for its anemia until the hemoglobin has sunk below 30 per cent. Below that value the organism increases the blood flow in order to secure to the tissues the normal amount of oxygen.

TABLE I.

Oxygen Unsaturation of the Venous Blood in a Patient with Severe Anemia.

Housewife, age 38 years.

Determination No.	Date.	Oxygen content of venous blood.			Hemoglobin (Haldane's method).	Calculated oxygen capacity (a).	Oxygen unsaturation (a–v).	Pulse.	Respirations per min.
		Sample 1.	Sample 2.	Average (v).					
	1918	*vol. per cent*	*vol. per cent*	*vol. per cent*	*per cent*	*vol. per cent*	*vol. per cent*		
1	July 12	4.40	4.00	4.20	41	7.58	3.38	98	22
2	" 18	3.54	3.64	3.59	38	7.03	3.44	96	20
3	Aug. 3	3.04		3.04	40	7.41	4.37	96	22
4	" 14	1.62	1.34	1.48	44	8.15	6.67	92	22
5	" 22	2.17	2.53	2.35	44	8.15	5.80	90	20
6	" 25	3.08	3.08	3.08	44	8.15	5.07	90	22
7	Sept. 9	2.53		2.53	44	8.15	5.62	90	22
8	" 14	1.75	2.21	1.98	44	8.15	6.17	92	20
9	" 21	1.74	2.20	1.97	48	8.88	6.91	86	20
10	Oct. 5	3.96	3.96	3.96	50	9.25	5.29	90	18
11	" 19	4.01	4.93	4.47	53	9.82	5.35	90	16

TABLE II.

Oxygen Unsaturation of the Venous Blood in a Patient with Severe Anemia.

Housewife, age 41 years.

Determination No.	Date.	Oxygen content of venous blood.			Hemoglobin (Haldane's method).	Calculated oxygen capacity (a).	Oxygen unsaturation (a–v .	Pulse.	Respirations per min.
		Sample 1.	Sample 2.	Average (v).					
	1918	*vol. per cent*	*vol. per cent*	*vol. per cent*	*per cent*	*vol. per cent*	*vol. per cent*		
1	Aug. 29	4.63	5.54	5.09	55	10.20	5.11	66	18
2	Sept. 3	6.46	6.46	6.46	75	13.87	7.40	60	16

TABLE III.

Oxygen Unsaturation of the Venous Blood in a Patient with Severe Anemia from Hematemesis.

Male, engineer, age 20 years.

Determination No.	Date.	Oxygen content of venous blood.			Hemoglobin (Haldane's method).	Calculated oxygen capacity (a).	Oxygen unsaturation (a–v).	Pulse.	Respirations per min.
		Sample 1.	Sample 2.	Average (v).					
	1918	*vol. per cent*	*vol. per cent*	*vol. per cent*	*per cent*	*vol. per cent*	*vol. per cent*		
1	Oct. 29	1.19		1.19	34	6.30	5.11	72	24
2	Nov. 5	1.27	1.05	1.16	32	5.93	4.77	80	18
3	" 14	2.20	2.20	2.20	43	7.96	5.76	62	18
4	Dec. 3	1.70	2.15	1.93	44	8.15	6.22	84	18
5	" 15	3.32		3.32	50	9.25	5.93	90	22

TABLE IV.

Oxygen Unsaturation of the Venous Blood in a Patient with Severe Anemia from Metrorrhagia.

Housewife, age 47 years.

Determination No.	Date.	Oxygen content of venous blood.			Hemoglobin (Haldane's method).	Calculated oxygen capacity (a).	Oxygen unsaturation (a–v).	Pulse.	Respirations per min.
		Sample 1.	Sample 2.	Average (v).					
	1918	*vol. per cent*	*vol. per cent*	*vol. per cent*	*per cent*	*vol. per cent*	*vol. per cent*		
1	Aug. 3	2.82		2.82	50	9.25	6.43	86	26
2	" 13	4.86	5.66	5.26	55	10.17	4.91	86	24
3	" 19	3.95	3.95	3.95	60	11.09	7.14	88	24
4	" 27	5.84	6.76	6.40	63	11.65	5.25	86	20
5	Sept. 9	6.68	6.68	6.68	73	13.50	6.82	86	20

TABLE V.

Oxygen Unsaturation of the Venous Blood in a Patient with Severe Anemia from Hemorrhagic Colitis.

Male, workman, age 33 years.

Determination No.	Date.	Oxygen content of venous blood.			Hemoglobin (Haldane's method).	Calculated oxygen capacity (a).	Oxygen unsaturation (a–v).	Pulse.	Respirations per min.
		Sample 1.	Sample 2.	Average (v).					
	1918	*vol. per cent*	*vol. per cent*	*vol. per cent*	*per cent*	*vol. per cent*	*vol. per cent*		
1	Sept. 19	1.73	1.73	1.73	42	7.78	6.05	76	24
2	" 25	4.54		4.54	55	10.27	5.78	80	24
3	Oct. 8	4.75	4.99	4.87	55	10.27	5.40	68	20
4	" 31	5.92	5.92	5.92	75	13.87	7.95	76	20

TABLE VI.

Oxygen Unsaturation of the Venous Blood in a Patient with Severe Anemia from Miscarriage.

Housewife, age 26 years.

Determination No.	Date.	Oxygen content of venous blood.			Hemoglobin (Haldane's method).	Calculated oxygen capacity (a).	Oxygen unsaturation (a–v).	Pulse.	Respirations per min.
		Sample 1.	Sample 2.	Average (v).					
	1918	*vol. per cent*	*vol. per cent*	*vol. per cent*	*per cent*	*vol. per cent*	*vol. per cent*		
1	Nov. 19	2.21	2.21	2.21	26	4.81	2.60	100	24
2	Dec. 4	3.05		3.05	30	5.55	2.50	112	24
3	" 8	3.87	3.87	3.87	43	8.70	4.93	100	26

TABLE VII.

Oxygen Unsaturation of the Venous Blood in a Patient with Severe Anemia from Hematemesis.

Male, carpenter, age 39 years.

Determination No.	Date.	Oxygen content of venous blood.			Hemoglobin (Haldane's method).	Calculated oxygen capacity (a).	Oxygen unsaturation (a–v).	Pulse.	Respirations per min.
		Sample 1.	Sample 2.	Average (v).					
	1918	*vol. per cent*	*vol. per cent*	*vol. per cent*	*per cent*	*vol. per cent*	*vol. per cent*		
1	Sept. 17	3.56	3.56	3.56	42	7.78	4.22	60	15
2	" 24	3.11	3.57	3.34	50	9.25	5.91	60	14
3	Oct. 3	4.07	4.97	4.52	55	10.27	5.75	60	14
4	" 12	4.04	4.86	4.45	55	10.27	5.82	60	14
5	Nov. 11	4.22		4.22	55	10.27	6.05	72	14
6	" 14	6.30	6.70	6.50	58	10.73	4.23	60	15

TABLE VIII.

Oxygen Unsaturation of the Venous Blood in a Patient with Severe Anemia.

Housewife, age 44 years.

Determination No.	Date.	Oxygen content of venous blood.			Hemoglobin (Haldane's method).	Calculated oxygen capacity (a).	Oxygen unsaturation (a–v).	Pulse.	Respirations per min.
		Sample 1.	Sample 2.	Average (v).					
	1918	*vol. per cent*	*vol. per cent*	*vol. per cent*	*per cent*	*vol. per cent*	*vol. per cent*		
1	Nov. 14	2.20	3.10	2.65	46	8.52	5.97	80	20
2	Dec. 29	10.55	10.35	10.45	80	14.80	4.35	76	19

TABLE IX.

Oxygen Unsaturation of the Venous Blood in a Patient with Pernicious Anemia.

Male, porter, age 44 years.

Determination No.	Date.	Oxygen content of venous blood.			Hemoglobin (Haldane's method).	Calculated oxygen capacity (a).	Oxygen unsaturation (a-v).	Pulse.	Respirations per min.
		Sample 1.	Sample 2.	Average (v).					
	1918	*vol. per cent*	*vol. per cent*	*vol. per cent*	*per cent*	*vol. per cent*	*vol. per cent*		
1	Aug. 13	7.46	7.38	7.42	68	12.57	5.15	72	22
2	" 21	4.90	4.90	4.90	73	13.50	8.60	84	24
3	" 28	5.86	5.96	5.92	78	14.42	8.50	82	22
4	Sept. 3	5.82	5.82	5.82	80	14.78	8.86	82	20
5	" 10	5.42	5.42	5.42	80	14.78	9.36	72	18
6	Oct. 14	6.70		6.70	87	16.03	9.33	70	18
7	Dec. 14	9.85	10.41	10.13	93	17.20	7.07	80	16

TABLE X.

Oxygen Unsaturation of the Venous Blood in a Patient with Pernicious Anemia.

Male, cab driver, age 53 years.

Determination No.	Date.	Oxygen content of venous blood.			Hemoglobin (Haldane's method).	Calculated oxygen capacity (a).	Oxygen unsaturation (a-v).	Pulse.	Respirations per min.
		Sample 1.	Sample 2.	Average (v).					
	1918	*vol. per cent*	*vol. per cent*	*vol. per cent*	*per cent*	*vol. per cent*	*vol. per cent*		
1	Aug. 3	2.82	2.82	2.82	32	5.92	3.10	70	30
2	" 13	2.61	2.43	2.52	40	7.41	4.89	66	20
3	" 25	1.63	1.37	1.50	36	6.67	5.17	68	20
4	Sept. 3	2.06		2.06	27	5.00	2.94	84	24
5	" 10	2.17	1.69	1.93	34	6.30	4.37	70	20
6	" 23	2.65	2.19	2.42	47	8.70	6.28	66	20
7	Oct. 5	6.31	6.59	6.40	60	11.09	4.69	60	20
8	" 19	4.46		4.46	70	12.94	8.48	60	20
9	Nov. 18	4.98	4.90	4.94	72	13.31	8.37	64	18
10	Dec. 16	7.17	6.27	6.72	74	13.58	6.86	60	20

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THE STREPTOCOCCI OF EQUINES.*

By F. S. JONES, V.M.D.

(*From the Department of Animal Pathology of The Rockefeller Institute for Medical Research, Princeton, N. J.*)

PLATE 12.

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INTRODUCTION.

Among the domestic animals no species is apparently more susceptible to infection with streptococci than the horse. In at least two diseases of the respiratory tract these microorganisms play a considerable part either as primary or secondary invaders. Streptococci are also responsible for many wound infections in horses and particularly in foals. Aside from a specific relationship to equine diseases, the relationship of the pathogenic streptococci found in horses to those of man is worthy of careful study.

Schütz (1) and Chantemesse and Delamotte (2) observed in the pneumonic areas of the lungs of horses suffering from influenza diplococci which when cultivated in bouillon developed as chains. Throughout the literature these streptococci are frequently referred to as the diplococcus or bicoccus of pneumonia.

Among the earlier workers Schütz (3), Baruchello (4), Sand and Jensen (5), and Poels (6) isolated or observed long chained streptococci in the nasal discharges and pharyngeal abscesses of horses suffering from strangles (adenitis equorum).

The earlier workers described the morphologic and cultural characters as they occurred on a few simple media such as agar, coagulated serum, and in bouillon and they attempted to show specific differences between these two streptococci. Their pathogenicity for mice and rabbits was clearly pointed out.

Galtier and Violet (7) also succeeded in isolating cocci in chains from the lungs of horses which had died of influenza. They observed that the length of chains varied widely in different tissues and cultures. In attempting to ascertain the source of the organism, they examined the nasal and pharyngeal mucosa

* This study was conducted during the war as a part of a study of infections occurring among horses, as a measure of military importance.

and feces of normal horses. They came to the conclusion that the specific streptococcus existed as a saprophyte in all these places. The characters on which the identifications were made were insufficient to separate streptococci into the various groups.

Lignières (8) isolated streptococci from the lungs, pleural fluid, liver, spleen, kidneys, and blood of horses dying of influenza. The cocci were usually ovoid and retained the stain by Gram's method. They grew as flocculi in bouillon and produced acid. Milk was usually coagulated in 24 to 48 hours. His cultures were pathogenic for mice and rabbits but guinea pigs were more resistant. 2 cc. of a bouillon culture injected subcutaneously into a horse produced an abscess. He compared Schütz's pneumonia diplococcus with Galtier and Violet's and his own streptococci and believed they were of the same species. He then undertook to show that Schütz's strangles streptococcus was identical with those isolated from influenza. Inasmuch as morphology and pathogenicity seemed to be the principal characters on which the comparisons were made, the results were not clear, although he regarded the streptococci as the same species. For a time Lignières was inclined to believe that streptococci were the cause of influenza but later he succeeded in isolating a rod-shaped organism of the septicemia hemorrhagica group which he claimed to be the agent. A horse inoculated with this culture died; not only was the bacterium recovered from the tissues but a streptococcus as well.

Ostertag (9) states that the nasal secretion of normal horses contains a mixture of bacteria including pus streptococci. He believed that these pus streptococci were identical with those found in influenza, but were not the cause of the disease since intranasal inoculation of material (nasal discharge) containing them failed to produce influenza.

During the next few years many writers discussed these organisms and their relationship to the diseases with which they were associated; in attempts to differentiate them, their virulence for mice and rabbits, the character of the growth in bouillon, and the length of chains proved as inconclusive as formerly. Thus Pfeiler (10) showed that the streptococci isolated from pyogenic conditions and pneumonia grew abundantly throughout bouillon. Bouillon cultures produce septicemia in mice. The strangles streptococcus grew more sparsely in bouillon and left the medium clear. Mice developed pyemia after subcutaneous injections. 50 to 60 cc. of a 24 hour bouillon culture of the pneumonia streptococcus injected intravenously into horses produced a severe reaction which usually terminated in pneumonia or pleuritis. In addition he found agglutinins for the streptococcus in the blood of horses suffering from influenza. He concluded that it was the cause of influenza.

Todd (11) described cocci occurring in chains of 30 to 60 individuals which he isolated from strangles abscesses. Microscopic examination of the nasal discharges usually revealed pairs and short chains. In bouillon they grew at the bottom of the tube, leaving the medium clear. Milk was coagulated in 6 or 8 days. Mice were exceedingly susceptible, rabbits more resistant. Horses when

inoculated subcutaneously with bouillon cultures developed abscesses. Cultures injected into the jugular vein caused only a slight general reaction. From his studies he does not believe that the causative streptococci are carried on the mucosa of the upper air passages of normal horses, but points out that they may be harbored for long periods after recovery.

Jensen (12) in discussing the specific prophylaxis and therapy of the streptococcic diseases of animals touches on the relationship of various streptococci in strangles, influenza, petechial fever, and wound infections. He considers that Holth (13) described a streptococcus (*Streptococcus equi*) which is the cause of strangles. It produces acid in dextrose, maltose, saccharose, and salicin but fails to ferment lactose, raffinose, inulin, or mannite, thus differing sharply from the diplococcus of influenza (*Brustseuchekokkus*) and the pyogenic streptococci. Jensen considers the etiology of influenza still in doubt but believes that the diplococci associated with it belongs to the *Streptococcus pyogenes* group. They differ from *Streptococcus equi* in their ability to ferment lactose and sorbite (Holth).

Bemelmans (14) brings forth a clinical observation to support the claim that strangles and influenza are not due to the same streptococcus. Thus cavalry horses that had recovered from strangles 9, 11, and 45 days previously came down with influenza. He argues that if the infections were caused by the same streptococcus the horses would not have been susceptible to a second attack within such a short period. Bemelmans also quotes Holth, who showed that the strangles organism was an encapsulated streptococcus which fermented dextrose, saccharose, maltose, and salicin but failed to break down lactose, raffinose, inulin, or glycerol.

Koch and Pokschischewsky (15) compared the human streptococcus, *erysipelatos*, with various strains of horse streptococci. The human types had been isolated by the writers. The horse streptococci, with the exception of three strains, had been isolated from cases of strangles in other laboratories. They had been grown on artificial media for long periods (1 to 5 years). The details concerning the more important biological characters are given in twenty instances. All the human strains fermented dextrose, saccharose, maltose, and mannite. Three failed to ferment lactose; the others acted upon this substance. All the horse strains produced acid in dextrose, saccharose, and maltose. Raffinose and mannite were not acted upon. Five strains fermented lactose; the others failed to do so. Both the human and equine streptococci produced hemolysis in blood agar plates. The equine types had a tendency to produce larger areas of hemolysis. The net acid production after several days incubation when measured with 0.05 N sodium hydroxide with phenolphthalein as indicator failed to show marked differences between the horse and human strains. Four strains of each type when grown in dextrose and titrated after maximum acid production had been reached averaged for the human +2.55 per cent and the equine +2.65. After studying the relative virulence of each type they concluded that the streptococcus of strangles is closely related to human *Streptococcus erysipelatos*. It is

interesting to observe that they assumed that the horse streptococci were all of one species whereas in reality there were two, one capable of fermenting lactose and the other unable to do so. The same holds true with the human types. The mannite fermentation by all the human strains was not regarded as of particular significance.

To Schofield (16) we are indebted for a clear description of the streptococci occurring in septic arthritis of foals in Canada. In fifteen cases he found streptococci in the affected joints. The cocci are described as growing in pairs on media other than bouillon. They stain by Gram's method. To determine hemolysis defibrinated rabbit blood, 1 cc., and agar, 10 cc., were poured into Petri dishes and allowed to congeal; the surface was then streaked with culture. Type II produced a clear space 5 to 6 mm. wide about the growth. The clear area extended to the bottom of the dish. In Type III he found that hemolysis took place immediately surrounding the growth but left a discolored zone between the hemolyzed area and the surrounding medium. Apparently the only differences between Types II and III were the size of the hemolytic area and the pathogenicity for rabbits. Type I is spoken of as the Schütz streptococcus but, since his protocols fail to show its presence in the joints or give any data concerning it and since no description of it is offered, it might as well have been omitted. Both Types II and III ferment dextrose, lactose, saccharose, and salicin but fail to ferment inulin, raffinose, dulcite, and mannite. He states: "The quantity of acid produced varied with the same strains under different conditions and with similar strains under the same conditions." It is pointed out, however, that no strain lost or acquired fermentative power.

Mathers (17) cultivated streptococci from the nasal discharges of horses suffering from influenza. In seven of twenty-two blood cultures streptococci developed. In addition he found them in exudate from the eye, the pleura, in purulent joints, and involved lymph nodes. Hemolytic streptococci were the only organisms occurring with any degree of regularity. On standard blood agar plates (1 cc. of defibrinated goat blood and 9 cc. of agar) the colonies were round, moist, and adherent. They were surrounded by a clear area of hemolysis 2 to 4 mm. in diameter. The morphology varied; pairs, chains of pairs, and chains were recorded. Many were Gram-negative. They fermented dextrose, lactose, saccharose, and salicin. Milk was acidulated but not coagulated. It is stated that many variations in the fermentative characters occurred. The streptococci were not highly pathogenic for rabbits but highly virulent for horses. Intranasal inoculation of a normal horse with "extracts" prepared from the nasal discharge of a horse suffering from influenza resulted in a "typical attack" of the disease. To test Gaffky and Lührs' (18) findings that influenza is caused by a filterable agent, a series of sixteen inoculations was made. The nasal exudate from diseased horses was suspended in salt solution and extracts of the pneumonic lung were prepared and both filtered. In no instance was influenza produced by the injection of filtrates. Intranasal sprays with the sediment obtained from 180 cc. of an ascitic dextrose broth culture produced a severe rhinitis

and pleuritis accompanied with fever and rapid pulse and respiration. The horse recovered in 18 days. Another horse was injected intravenously with sediment from the same amount of culture. It developed symptoms of influenza and died 8 days later. Autopsy revealed fibrinous pleuritis, bronchopneumonia, and evidences of septicemia.

Since the relationship of the strangles streptococcus with those associated with influenza seemed confused, it was considered advisable to study these streptococci with the methods now in use. In addition, the problem of ascertaining whether streptococci similar to those found in strangles and influenza were carried on the nasal mucosa or in the pharynx of horses presented itself. Studies were made on the following classes of horses: (1) normal horses (eastern) that had been in this vicinity for some time; (2) apparently normal horses that had recently been shipped from the West; (3) those suffering from strangles, influenza, rhinitis, and purulent conditions.

Streptococci of the Nasal Mucosa and Upper Pharynx of Normal Horses.

Sterile cotton swabs wound on bale wire, 15 and 30 cm. in length, were employed to obtain material. The shorter were used for the nasal passages and the longer for the pharynx. The same swab was used in both nostrils. In making preparations from the throat the mouth was held open with a speculum, the tongue depressed, and the swab brushed over the mucosa of the upper pharynx. Within 2 hours the swabs were agitated for a few seconds in tubes containing 10 cc. of sterile 0.9 per cent salt solution. Two or three loopfuls of suspension were inoculated into a tube containing 12 cc. of melted veal infusion agar (2 per cent) cooled to 45°C. and the whole was plated with 1 cc. of defibrinated horse blood. After 24 hours incubation at 38°C. subcultures were made from colonies resembling streptococci.

The results of the examinations of the thirty eastern horses may be summarized as follows:

Horses carrying non-hemolytic streptococci on the nasal mucosa	8
Horses carrying hemolytic streptococci on the nasal mucosa	0
Horses carrying non-hemolytic streptococci in the pharynx	6
Horses carrying hemolytic streptococci in the pharynx	18

The morphological and cultural characters of these streptococci are given in Tables I, II, and III. Inoculations from 18 hour broth cultures were made into tubes containing 13 cc. of fermented bouillon adjusted to +0.6 to +0.8 (phenolphthalein), to which had been added sufficient amounts of the carbohydrates and other substances to make a 1 per cent solution. Titrations were made after 5 days incubation at 38°C. An incubation period of this length was considered sufficient since the maximum acid production is usually reached within 36 hours. The amounts recorded in the tables represent the net acid production.[1] Litmus milk was likewise incubated for 5 days. On removal from the incubator if not coagulated the tubes were boiled for a few minutes. The figures under the column "Diameter of area of hemolysis" represent the diameter of hemolysis about the deep colonies after 24 hours incubation.

Smith and Brown (19) and Brown (20) describe two principal types of hemolysis. The alpha type is characterized by a partial hemolysis and greenish discoloration of the red cells immediately surrounding the deep colonies. This zone is succeeded by another clear band of partially hemolyzed cells. In the beta type the hemolytic area is sharply defined and clear, since the cells are completely hemolyzed. The beta type has been frequently observed among the horse streptococci but the alpha type has not been met with. A characteristic hemolytic zone has been noted with considerable frequency in the throat strains from horses. It surrounds both the surface and deep colonies as a clearly defined area varying in color from light orange to a delicate red. In certain instances there is a narrow clearer line between the colony and the larger discolored area. Microscopic examination reveals the presence of aggregates of unhemolyzed red cells throughout the lighter colored zone. The intensity of color appears to depend on the number of unhemolyzed cells. If the incubated plates are stored for 24 or 48 hours the color of the unhemolyzed cells has a tendency to deepen. When rabbit blood is used in the plate cultures the picture is not so striking. Colonies surrounded by the narrow clear line have been indicated

[1] The total acidity reached in the various test substances may be approximated by adding 0.7 per cent to the amounts given in the table.

in the tables as Type xa (Figs. 1 and 2), and those which showed only the partially hemolyzed area as Type x (Figs. 3 and 4).

Since all strains were Gram-positive, mention of this characteristic has been omitted from the tables.

The nasal strains fall into two groups when classified on the basis of lactose fermentation. In both the proportion of mannite-fermenting strains is high. The predominating type is a streptococcus which produces acid in dextrose, lactose, saccharose, maltose, man-

TABLE I.

Non-Hemolytic Streptococci from the Nasal Mucosa of Eastern Horses.

Strain No.	Grouping.	Growth in bouillon.	Litmus milk.	Production of acid in.							
				Dextrose.	Lactose.	Saccharose.	Maltose.	Raffinose.	Inulin.	Mannite.	Salicin.
				per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
N 5	L. C.*	Clear.	Coagulated on boiling.	3.0	2.6	2.4	2.4	0.0	0.0	2.6	1.9
N 8	"	"	" " "	3.4	2.8	2.8	2.5	0.0	0.0	2.5	2.6
N 9	"	Turbid.	" " "	2.7	2.8	2.8	2.4	0.0	0.0	1.9	2.1
N 10	"	"	" " "	2.5	2.3	2.4	2.2	0.1	0.2	1.9	2.4
N 11	"	Clear.	" " "	2.3	2.1	0.0	2.2	0.0	0.0	1.7	2.3
N 13	"	"	" " "	2.5	2.4	2.3	2.4	0.2	0.0	2.7	2.0
N 15	"	"	" " "	3.2	3.5	2.2	3.0	0.2	0.1	2.7	2.5
N 19	"	"	" " "	3.5	3.5	3.5	3.0	0.0	0.0	2.5	2.3
N 6	Pairs and S. C.	Turbid.	Unchanged.	1.7	0.2	1.4	1.5	0.2	0.2	0.2	1.6
N 16	" " "	"	"	1.8	0.0	1.8	1.8	0.1	0.0	0.0	1.9
N 3	L. C.	Clear.	"	3.0	0.1	2.7	3.0	2.8	0.1	2.7	3.2
N 4	"	"	"	2.4	0.1	2.4	2.4	0.1	0.1	2.5	2.4

* L. C. indicates chains of 20 or more elements; threads of 8 to 20 are indicated as M. C., and those made up of 6 or 8 cocci as S. C.

nite, and salicin. One strain failed to ferment saccharose. None of these streptococci proved pathogenic for mice when 0.1 cc. of a 24 hour bouillon culture was injected into the peritoneal cavity.

Six of the seven strains of non-hemolytic streptococci from the pharynx failed to ferment lactose. They differed from the nasal types in their inability to ferment mannite. All except one attacked raffinose or inulin. The hemolytic streptococci possessed similar fermentative characters, although the proportion of lactose-fermenting

and non-lactose-fermenting strains was nearly equal. They too were capable of breaking down raffinose or inulin. Acid production in mannite was not infrequent but always occurred in combination with raffinose or inulin fermentation. Strain P 14 differed sharply from the others in its cultural characters and its pathogenic properties. This species was extremely virulent for mice and rabbits. The others failed to produce ill effects when 0.1 cc. of a 24 hour bouillon culture was injected into the peritoneal cavity of mice, or when 1 cc. was injected intravenously into rabbits.

TABLE II.

Non-Hemolytic Streptococci from the Pharynx of Eastern Horses.

Strain No.	Grouping.	Growth in bouillon.	Litmus milk.	Production of acid in.							
				Dextrose.	Lactose.	Saccharose.	Maltose.	Raffinose.	Inulin.	Mannite.	Salicin.
				per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
P 41	Pairs and S. C.	Turbid.	Unchanged.	4.3	0.0	3.7	4.0	3.5	3.4	0.0	3.1
P 45	L. C.	"	"	3.0	0.1	2.4	2.6	2.5	3.0	0.2	3.0
P 13	Pairs.	Clear.	"	3.8	0.1	2.6	3.0	0.3	2.6	0.1	2.7
P 42	L. C.	Turbid.	"	4.8	0.0	4.4	4.5	0.0	4.7	0.1	4.8
P 52	"	"	"	3.2	0.1	3.0	2.9	0.1	2.7	0.1	2.8
P 5	M. C.	"	"	4.7	0.1	4.5	3.2	0.1	0.2	0.1	3.9
P 36	"	"	Coagulated on boiling.	3.4	2.9	3.5	3.6	2.5	3.6	0.1	3.5

From these studies several points stand out clearly. The bulk of the nasal flora is made up of non-hemolytic streptococci which ferment mannite. In the pharynx both hemolytic and non-hemolytic streptococci which ferment raffinose and inulin predominate. Strains which fail to produce acid in lactose are frequent in both regions.

Since the preceding results had established sufficiently the streptococcic flora of normal horses which had been in the East for some time, studies were begun on horses which had been recently shipped from the West. These animals were apparently normal when the cultures were made. Horses were examined over a period of 5 months. It may be assumed that they had been exposed to influenza and other diseases at various shipping points. Some of their fellows were

TABLE III.

Hemolytic Streptococci from the Pharynx of Eastern Horses.

Strain No.	Grouping.	Growth in bouillon.	Litmus milk.	Production of acid in.								Diameter of area of hemolysis.	Type of hemolysis.
				Dextrose.	Lactose.	Saccharose.	Maltose.	Raffinose.	Inulin.	Mannite..	Salicin.		
				per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*mm.*	
P 3	L. C.	Clear.	Unchanged.	2.1	0.0	1.9	2.0	1.7	1.9	0.0	2.0	3.0–3.5	Beta.
P 16	"	"	"	2.7	0.0	2.8	2.8	2.4	2.8	0.0	3.3	4.0–5.0	x
P 19	"	Turbid.	"	2.8	0.1	2.5	2.8	2.3	3.2	0.0	2.8	3.0	x
P 20	"	Clear.	"	1.3	0.0	1.4	1.5	1.9	1.3	0.0	1.8	3.5–4.0	Beta.
P 30	"	"	"	2.8	0.0	2.6	2.6	2.2	1.7	0.0	2.2	1.0	x
P 39	M. C.	"	"	2.8	0.0	2.2	2.1	2.2	1.8	0.0	0.6	2.5–3.0	x
P 43	Pairs and S. C.	Turbid.	"	4.0	0.0	3.7	4.0	3.5	3.4	0.0	0.8	3.5–4.0	x
P 11	" " "	"	"	3.7	0.3	3.7	3.5	2.9	1.7	3.3	3.3	2.0–2.5	x
P 24	M. C.	"	"	2.6	0.0	2.3	2.6	0.0	2.2	0.0	2.4	2.5–3.0	Beta.
P 47	Pairs and S. C.	Clear.	"	2.4	0.0	2.4	2.2	0.0	2.4	0.0	2.4	2.5–3.0	x
P 26	S. C.	Turbid.	"	4.6	0.0	4.2	4.0	0.0	4.3	2.2	4.2	2.0	x
P 22	L. C.	Clear.	Coagulated on boiling.	1.7	1.3	1.7	1.8	1.4	1.8	0.0	1.8	3.0–4.0	x
P 35	"	"	" " "	3.8	3.5	3.8	3.5	0.6	3.7	0.2	4.0	3.0–3.5	x
P 38	M. C.	"	" " "	2.9	2.7	2.9	2.7	2.5	2.1	0.0	3.0	2.5–3.0	Beta.
P 51	L. C.	"	" " "	3.6	2.1	3.4	3.1	2.7	2.9	0.0	2.6	4.5–5.0	x
P 23	Pairs and S. C.	Turbid.	Acid.	2.8	2.6	3.0	3.1	2.5	2.3	2.8	2.9	2.5–3.0	Beta.
P 1	Pairs.	"	Coagulated on boiling.	3.4	3.1	3.2	3.3	2.9	0.0	3.1	2.9	4.0–5.0	xa
P 6	" and S. C.	"	" " "	3.1	2.7	3.1	3.2	2.6	0.0	2.6	2.9	4.0–5.0	xa
P 53	" " "	"	Acid.	3.8	3.2	3.7	3.8	3.2	0.0	2.9	3.8	3.0–3.5	x
P 40	M. C.	"	Firmly coagulated.	4.7	4.5	5.2	5.2	4.2	0.2	0.0	4.1	6.0–7.0	xa
P 14	"	Clear.	Coagulated on boiling.	3.4	2.8	3.1	3.3	0.0	0.0	0.0	2.4	3.0–3.5	Beta.

suffering from influenza when the cultures were made. In all probability many had been exposed repeatedly. It was impossible to observe them after they had left the sales stables; some may have come down later.

The following summarizes the results of the nasal and pharyngeal examinations of the twenty-three western horses:

Horses carrying non-hemolytic streptococci on the nasal mucosa.... 8
Horses carrying hemolytic streptococci on the nasal mucosa..... 8
Horses carrying non-hemolytic streptococci in the pharynx..... 11
Horses carrying hemolytic streptococci in the pharynx..... 11

TABLE IV.

Non-Hemolytic Streptococci from the Nasal Mucosa of Western Horses.

Strain No.	Grouping.	Growth in bouillon.	Litmus milk.	Production of acid in.							
				Dextrose.	Lactose.	Saccharose.	Maltose.	Raffinose.	Inulin.	Mannite.	Salicin.
				per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
N 26	L. C.	Turbid.	Coagulated, not firmly.	4.1	3.0	3.9	3.6	0.0	0.0	2.7	3.5
N 40	"	Clear.	" on boiling.	3.5	3.7	3.7	3.3	0.0	0.2	2.7	2.6
N 44	"	"	" " "	2.9	2.5	3.4	3.1	0.0	0.0	2.9	2.5
N 45	"	"	" " "	3.9	3.2	3.0	3.4	0.1	0.1	3.0	4.1
N 27	Pairs and S. C.	Turbid.	" " "	3.2	3.0	3.1	3.2	0.0	3.2	0.0	0.0
N 41	" " "	"	" " "	3.6	3.4	3.8	3.8	0.1	3.3	0.1	0.0
N 24	M. C.	"	" " "	3.7	3.1	3.7	3.5	0.0	0.0	0.0	3.0
N 33	"	Clear.	Unchanged.	3.3	0.1	3.4	3.4	2.7	3.1	0.0	3.2
N 46	"	"	"	4.2	0.1	3.9	3.6	3.4	0.0	3.0	5.0

An outstanding feature is the presence of hemolytic streptococci in the lower nasal passages of 30 per cent of the western horses. Streptococci of this type were not found in the same region in the eastern horses. The morphological and cultural characters are given in Tables IV to VII.

It is interesting to observe that the non-hemolytic streptococci from the nasal mucosa are similar in both eastern and western horses. Mannite fermenters make up the majority of the flora, although raffinose and inulin fermentation was observed more frequently. A striking difference in the flora of the two classes of horses is the pres-

TABLE V.

Hemolytic Streptococci from the Nasal Mucosa of Western Horses.

Strain No.	Grouping.	Growth in bouillon.	Litmus milk.	Production of acid in.								Diameter of area of hemolysis.	Type of hemolysis.
				Dextrose.	Lactose.	Saccharose.	Maltose.	Raffinose.	Inulin.	Mannite.	Salicin.		
				per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*mm.*	
N 20	M. C.	Turbid.	Coagulated on boiling.	3.8	3.0	3.6	3.6	0.0	0.0	0.0	3.4	3.5–4.0	Beta.
N 25	L. C.	"	" " "	4.2	4.0	4.4	4.3	0.1	0.0	0.0	3.4	3.0–3.5	"
N 28	"	"	" " "	4.3	3.8	4.5	4.2	0.0	0.2	0.0	3.0	2.5–3.0	"
N 29	"	Clear.	" " "	4.6	3.2	4.2	4.2	0.0	0.0	0.0	3.4	3.5–4.0	"
N 38	M. C.	Turbid.	" " "	4.2	3.7	3.8	4.7	0.0	0.1	0.1	3.8	3.5–4.0	"
N 42	"	"	" " "	4.8	3.8	4.0	3.8	0.0	0.0	0.0	4.4	3.0	"
N 47	S. C.	Clear.	" " "	4.2	3.6	4.1	4.1	0.0	0.0	0.0	3.6	3.5–4.0	"
N 48	M. C.	"	" " "	4.1	3.6	4.0	4.0	0.2	0.2	0.0	3.5	3.5–4.0	"

TABLE VI.

Non-Hemolytic Streptococci from the Pharynx of Western Horses.

Strain No.	Grouping.	Growth in bouillon.	Litmus milk.	Production of acid in.							
				Dextrose.	Lactose.	Saccharose.	Maltose.	Raffinose.	Inulin.	Mannite.	Salicin.
				per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
P 48	L. C.	Turbid.	Unchanged.	3.5	0.0	3.0	3.3	3.0	3.2	0.2	3.1
P 67	S. C.	"	"	2.7	0.1	2.6	2.7	3.1	4.0	0.0	3.6
P 73	L. C.	"	"	3.9	0.0	3.8	4.0	3.2	3.4	0.0	3.3
P 74	"	"	"	4.2	0.0	3.7	3.8	3.5	4.3	0.1	3.9
P 84	S. C.	"	"	4.0	0.0	4.2	4.2	3.5	3.3	4.0	4.0
P 64	L. C.	Clear.	"	7.2	0.0	6.4	5.7	5.6	0.0	0.0	5.4
P 75	"	Turbid.	"	3.8	0.0	4.0	4.1	0.0	3.7	0.0	3.5
P 76	M. C.	"	"	3.3	0.2	3.4	3.1	0.0	3.3	0.0	2.7
P 58	Pairs and S. C.	"	Firmly coagulated.	4.4	3.6	3.5	3.4	3.3	2.9	0.0	3.0
P 60	L. C.	"	Coagulated on boiling.	3.6	3.0	3.6	3.3	3.5	3.7	0.0	3.6
P 69	M. C.	"	" " "	2.9	2.3	3.3	2.9	2.2	2.8	0.0	1.7
P 72	L. C.	"	" " "	3.8	3.0	3.9	4.3	3.4	3.2	0.0	3.7
P 78	"	Clear.	" not firmly.	4.1	3.5	3.1	3.8	2.9	4.1	0.0	3.2
P 83	S. C.	Turbid.	" on boiling.	4.1	3.2	4.0	3.9	3.4	3.8	0.1	3.6

ence of pathogenic hemolytic streptococci (*Streptococcus pyogenes*) on the nasal mucosa of the western horses (Table V). These streptococci produced acid in dextrose, lactose, saccharose, maltose, salicin, and milk. The other substances were not fermented. Mice, when injected intraperitoneally with 0.1 cc. of a 24 hour bouillon culture, developed septicemia and died within 24 or 48 hours. Rabbits when injected intravenously with 1 cc. of culture were much more resistant;

TABLE VII.

Hemolytic Streptococci from the Pharynx of Western Horses.

Strain No.	Grouping.	Growth in bouillon.	Litmus milk.	Production of acid in.								Diameter of area of hemolysis.	Type of hemolysis.
				Dextrose.	Lactose.	Saccharose.	Maltose.	Raffinose.	Inulin.	Mannite.	Salicin.		
				per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	mm.	
P 55	L. C.	Clear.	Coagulated on boiling.	4.0	3.3	3.9	3.6	0.1	0.0	0.0	4.0	3.0–3.5	Beta.
P 56	S. C.	"	Acid.	3.8	3.4	4.0	4.0	0.0	0.0	0.0	3.4	3.0–3.5	"
P 61	L. C.	Turbid.	Coagulated on boiling.	4.2	3.3	4.0	4.4	0.0	0.1	0.0	4.2	2.5–3.0	"
P 62	"	Clear.	" " "	4.4	3.6	4.1	4.5	0.1	0.2	0.0	3.6	3.0–3.5	"
P 63	M. C.	"	" " "	4.2	3.0	3.9	4.3	0.0	0.0	0.0	4.1	3.0–3.5	"
P 79	L. C.	Turbid.	Coagulated, not firmly.	4.6	3.9	4.3	4.5	0.1	0.0	0.0	5.0	3.0–3.5	"
P 80	S. C.	Clear.	" " "	5.3	4.3	4.9	5.3	0.0	0.0	0.0	4.6	3.5–4.0	"
P 81	L. C.	"	" " "	5.3	3.9	5.1	5.3	0.0	0.1	0.1	4.9	3.5–4.0	"
P 57	S. C.	Turbid.	Unchanged.	3.9	0.0	3.9	4.0	3.3	3.3	3.6	3.7	3.5–4.0	x
P 68	"	"	"	3.8	0.0	4.0	4.2	3.0	2.8	2.9	3.7	6.0–7.0	xa
P 66	L. C.	Clear.	"	2.0	0.0	1.8	1.9	2.4	2.0	0.0	1.7	2.5–3.0	xa
P 82	M. C.	"	Coagulated on boiling.	5.0	3.9	4.2	4.4	0.0	5.5	2.6	3.7	3.0–3.5	Beta.
P 65	L. C.	Turbid.	" " "	5.2	3.8	6.0	5.2	4.0	0.0	0.0	4.1	3.5–4.0	"

many became emaciated and showed severe temperature reactions. Some recovered while others developed purulent arthritis. Larger doses of culture usually produced septicemia.

The same groups which were encountered in the pharyngeal flora of eastern horses were observed in the western horses. Among the non-hemolytic streptococci raffinose- and inulin-fermenting types predominated. Strains which failed to ferment lactose were frequent.

The horses from the West carried *Streptococcus pyogenes* in the throat in eight instances. The other five strains given in Table VII are raffinose or inulin fermenters which may or may not act upon mannite. They are similar to those observed in Table III. Three streptococci of the *pyogenes* type from the pharynx possessed the same pathogenic properties for rabbits and mice as those found on the nasal mucosa.

Pathogenic Streptococci of the Nasal Mucosa and Upper Pharynx of Diseased Horses.

Pathogenic streptococci have been isolated from horses suffering from acute diseases of the respiratory tract (influenza, rhinitis, pharyngitis, and strangles) and from infected wounds. The material was obtained by brushing the affected surfaces with a sterile swab. Salt solution suspensions were then made. Plates were prepared within a few hours.

Among twenty-four cases of influenza hemolytic streptococci were found in considerable numbers in the nasal discharge from twenty-two. The plate cultures from the pharynx were positive in eight cases. When conjunctivitis (pink eye) was an accompanying factor streptococci were obtained in practically pure culture from the ocular exudate. It was possible to obtain material from six cases of strangles. Pus from various acute and chronic purulent conditions was suspended in salt solution from which plate cultures were made. Streptococci were frequently found in pure culture. Table VIII indicates the cultural characteristics of these streptococci.

It will be observed that the streptococci fall into two broad groups. The larger group, composed of streptococci from influenza, strangles (three cases), and all but one abscess are of *Streptococcus pyogenes* type. They produce acid in dextrose, lactose, saccharose, maltose, and milk but fail to acidulate raffinose, inulin, or mannite. The smaller group, *Streptococcus equi*, includes the non-lactose-fermenting strains which do not produce acid in raffinose, inulin, mannite, or milk. These were found in influenza and strangles, rhinitis and pharyngitis, and once in an abscess.

Both types are especially virulent for mice. Rabbits are more resistant. 1 cc. of a 24 hour bouillon culture of certain strains may

TABLE VIII.

Pathogenic Streptococci.

Strain No.	Source.	Grouping.	Growth in bouillon.	Litmus milk.	Production of acid in.								Diameter of area of hemolysis.
					Dextrose.	Lactose.	Saccharose.	Maltose.	Raffinose.	Inulin.	Mannite.	Salicin.	
					per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*mm.*
In. 14	Nasal exudate; influenza.	L. C.	Clear.	Coagulated on boiling.	3.5	2.8	3.5	3.6	0.0	0.0	0.0	3.5	3.5–4.0
In. 15	" " "	"	"	" " "	3.7	2.9	3.8	3.6	0.0	0.1	0.0	3.7	3.0–3.5
In. 19	" " "	"	Turbid.	" " "	4.2	3.3	3.9	4.4	0.1	0.0	0.0	4.3	3.0–3.5
In. 20	" " "	Pairs and S. C.	Clear.	" " "	4.0	3.4	4.2	4.1	0.0	0.0	0.0	3.9	4.5–5.0
In. 22	Pharynx; "	L. C.	"	" " "	3.9	3.7	3.5	3.7	0.0	0.0	0.0	3.9	3.5–4.0
In. 25	Nasal exudate; "	"	"	" " "	5.0	3.8	4.7	4.2	0.0	0.0	0.0	4.3	3.0–3.5
In. 26	" " "	"	"	" " "	4.1	3.0	4.0	3.8	0.0	0.0	0.0	3.5	3.0–3.5
In. 31	" " "	"	Turbid.	" " "	4.0	3.4	4.1	4.0	0.0	0.0	0.0	3.6	3.5–4.0
In. 32	" " "	"	"	" " "	5.0	4.1	4.7	5.0	0.0	0.0	0.1	4.1	3.5–4.0
In. 36	Pharynx; "	M. C.	"	" " "	4.3	3.5	3.9	4.2	0.0	0.0	0.0	3.4	3.0–3.5
In. 38	Nasal exudate; "	S. C.	Clear.	" " "	4.2	3.5	4.2	4.4	0.0	0.0	0.0	4.3	3.0–3.5
In. 42	Exudate from eye; "	M. C.	"	" " "	4.2	3.1	4.1	4.4	0.0	0.0	0.0	3.3	2.5–3.0
In. 43	" " " "	"	Turbid.	" " "	4.6	3.6	4.2	4.8	0.1	0.0	0.2	4.1	2.5–3.0
In. 46	Nasal exudate; "	"	Clear.	" " "	4.1	3.1	4.0	4.0	0.0	0.1	0.0	3.4	2.5–3.0
In. 47	" " "	S. C.	"	" " "	4.3	3.7	4.1	4.0	0.0	0.0	0.0	3.4	2.5–3.0
In. 48	Pharynx; "	Pairs and S. C.	Turbid.	" " "	4.2	3.1	3.7	4.2	0.0	0.0	0.1	3.0	3.0–3.5
In. 49	Nasal exudate; "	M. C.	"	" " "	4.2	2.9	4.0	4.3	0.0	0.0	0.0	4.0	3.5–4.0
In. 50	" " "	S. C.	"	" " "	4.3	2.9	3.7	4.3	0.0	0.0	0.0	4.0	3.5–4.0
In. 52	Pharynx; "	M. C.	"	" " "	4.1	2.8	4.1	4.0	0.0	0.1	0.0	3.8	4.0–4.5
In. 53	" "	Pairs and S. C.	"	"	4.3	3.3	3.8	4.3	0.1	0.0	0.0	3.9	3.0–3.5

In. 54	Nasal exudate; influenza.	M. C.	Turbid.	Coagulated on boiling.	4.1	3.1	3.6	4.2	0.0	0.1	0.0	3.4	3.5–4.0
In. 55	" " "	L. C.	Clear.	" " "	4.3	3.6	4.2	4.2	0.0	0.0	0.0	3.5	3.5–4.0
In. 57	" " "	Pairs and S. C.	Turbid.	" " "	4.4	3.0	4.1	4.0	0.1	0.1	0.0	4.0	3.0–3.5
In. 58	" " "	" " "	"	" " "	4.6	3.0	4.2	4.3	0.0	0.0	0.0	3.8	3.5–4.0
In. 59	" " "	" " "	"	" " "	4.2	3.4	4.1	4.3	0.0	0.0	0.0	4.0	3.0–3.5
In. 60	" " "	S. C.	Clear.	" " "	4.1	3.0	3.7	3.9	0.0	0.0	0.0	3.2	2.0–2.5
In. 62	" " "	M. C.	Turbid.	" " "	3.7	3.3	3.5	3.5	0.0	0.0	0.0	3.2	3.0–3.5
In. 63	" " "	"	"	" " "	3.7	2.9	3.7	3.4	0.0	0.0	0.0	3.2	3.0–3.5
In. 64	" " "	L. C.	"	Partially coagulated on boiling.	4.0	3.1	3.0	4.1	0.0	0.0	0.0	3.2	3.0–3.5
In. 66	" " "	"	"	Acid.	3.9	3.1	3.8	3.9	0.0	0.1	0.0	3.4	2.0–2.5
In. 69	Pharynx; "	"	"	Coagulated on boiling.	4.0	3.7	4.1	3.5	0.0	0.0	0.0	3.6	2.0–2.5
In. 70	"	"	"	" " "	3.2	3.2	3.1	3.8	0.0	0.0	0.1	3.3	2.0–2.5
In. 71	Nasal exudate; "	"	Clear.	" " "	4.0	3.3	4.1	4.0	0.0	0.1	0.0	3.7	2.0–2.5
In. 76	" " "	S. C.	Turbid.	" " "	3.2	3.0	3.0	3.1	0.0	0.1	0.1	2.9	2.5–3.0
In. 77	Pharynx; "	M. C.	Clear.	" " "	4.1	3.6	3.3	3.9	0.1	0.1	0.1	3.4	3.0–3.5
In. 79	Nasal exudate; "	"	"	" " "	4.4	3.6	4.1	3.8	0.1	0.2	0.1	3.0	2.5–3.0
In. 81	" " "	"	Turbid.	" " "	3.7	2.7	3.3	3.7	0.1	0.1	0.1	3.2	2.5–3.0
In. 84	" " "	L. C.	Clear.	" " "	3.7	2.9	3.6	3.6	0.0	0.1	0.0	2.9	3.5–4.0
In. 88	" " "	"	Turbid.	" " "	3.1	2.9	3.1	3.4	0.0	0.1	0.0	2.3	2.5–3.0
In. 90	" " "	M. C.	"	" " "	3.8	3.1	3.6	3.5	0.1	0.0	0.0	2.7	2.5–3.0
Str. 1	" " strangles.	"	"	" " "	4.4	3.5	4.1	4.2	0.1	0.1	0.0	3.8	3.0–3.5
Str. 20	" " "	"	"	Acid.	3.6	3.2	3.4	3.8	0.0	0.0	0.0	3.5	3.0–3.5
Str. 22	Abscess; "	L. C.	Clear.	"	3.7	2.5	3.3	3.5	0.0	0.0	0.0	3.4	3.0–3.5
HA 2	"	S. C.	"	Coagulated on boiling.	3.7	2.9	3.7	3.6	0.0	0.0	0.0	3.1	2.5–3.0
HA 3	"	M. C.	Turbid.	" " "	4.2	3.3	3.8	3.3	0.0	0.1	0.0	4.1	3.5–4.0
HA 5	"	"	"	" " "	4.5	3.5	3.8	4.3	0.0	0.0	0.0	3.5	3.0–3.5
HA 6	"	S. C.	"	" " "	4.6	3.7	3.9	4.3	0.0	0.1	0.0	3.6	3.5–4.0
HA 7	"	M. C.	Clear.	" " "	4.3	3.3	3.6	4.1	0.0	0.0	0.0	3.9	3.5–4.0
HA 10	"	S. C.	Turbid.	" " "	4.9	3.7	4.6	4.4	0.1	0.0	0.0	3.5	2.0– .5
HA 13	"	L. C.	Clear.	" " "	3.3	3.0	3.4	3.3	0.0	0.0	0.0	3.1	3.5–4.0

TABLE VIII—*Concluded.*

Strain No.	Source.	Grouping.	Growth in bouillon.	Litmus milk.	Production of acid in.								Diameter of area of hemolysis.
					Dextrose.	Lactose.	Saccharose.	Maltose.	Raffinose.	Inulin.	Mannite.	Salicin.	
					per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*mm.*
HA 16	Placenta; abortion.	M. C.	Turbid.	Coagulated on boiling.	3.6	3.1	3.6	3.6	0.0	0.0	0.0	3.1	3.0–3.5
HA 17	Metritis.	"	"	" " "	4.2	3.2	3.7	3.9	0.0	0.0	0.0	3.3	3.0–3.5
HA 19	Abscess.	"	"	" " "	3.9	3.1	2.7	3.9	0.1	0.0	0.0	2.8	2.5–3.0
N 23	Nasal exudate; rhinitis.	"	"	" " "	4.4	3.6	3.8	4.0	0.0	0.0	0.1	3.9	3.0–3.5
In. 2	" " influenza.	S. C.	Clear.	Unchanged.	4.3	0.0	3.9	4.4	0.1	0.0	0.0	3.4	3.0–3.5
In. 3	" " "	"	Turbid.	"	4.5	0.1	4.1	4.3	0.1	0.1	0.0	3.3	3.5–4.0
In. 5	Pharynx; "	M. C.	"	"	4.4	0.1	4.0	4.4	0.0	0.0	0.0	3.6	3.0–3.5
In. 6	" "	S. C.	"	"	4.4	0.1	3.9	4.3	0.0	0.0	0.0	3.3	3.0–3.5
In. 9	Nasal exudate; "	"	Clear.	"	4.3	0.0	4.4	4.5	0.2	0.2	0.2	4.4	1.5–2.0
In. 10	" " "	"	"	"	4.7	0.2	4.4	4.7	0.0	0.0	0.0	3.9	1.5–2.0
In. 11	Exudate from eye; "	"	Turbid.	"	4.5	0.0	4.1	4.4	0.0	0.0	0.0	3.4	3.0–3.5
In. 12	" " " "	"	Clear.	"	4.4	0.1	4.1	4.5	0.0	0.1	0.0	3.4	3.5–4.0
In. 73	" " " "	M. C.	Turbid.	"	4.8	0.0	4.5	4.7	0.0	0.0	0.1	4.2	2.0–2.5
In. 86	" " " "	L. C.	"	"	4.1	0.0	3.6	4.6	0.0	0.1	0.0	3.1	3.5–4.0
Str. 6	Nasal exudate; strangles.	S. C.	"	"	4.2	0.0	4.0	3.8	0.0	0.0	0.0	3.9	2.0–2.5
Str. 9	" " "	M. C.	"	"	4.2	0.0	3.7	4.4	0.0	0.0	0.0	3.5	3.0–3.5
Str. 12	Abscess; "	"	"	"	4.0	0.0	3.7	4.1	0.1	0.0	0.0	3.2	3.0–3.5
Str. 16	" "	S. C.	"	"	3.9	0.1	3.1	3.7	0.1	0.2	0.2	3.6	3.5–4.0
Str. 18	" "	M. C.	"	"	3.1	0.0	3.5	3.2	0.0	0.0	0.0	2.6	3.5–4.0
Str. 23	" "	Pairs and S. C.	"	"	3.8	0.1	3.8	4.0	0.1	0.1	0.1	3.0	3.0–3.5
HA 8	"	M. C.	Clear.	"	3.4	0.1	3.4	3.5	0.0	0.0	0.0	3.5	2.0–2.5
N 23A	Nasal exudate; rhinitis.	"	Turbid.	"	4.6	0.1	4.6	4.6	0.1	0.1	0.1	4.6	3.0–3.5
P 70	Pharyngitis.	L. C.	"	"	4.8	0.0	4.3	4.8	0.1	0.1	0.0	3.4	3.0

cause septicemia but usually produces severe febrile disturbances or causes localization in joints. Arthritis is the most frequent result of intravenous injections.

The colonies of *Streptococcus pyogenes* are round, slightly raised, translucent, and rarely exceed 1.5 mm. in diameter. The hemolytic area is of the beta type and is pronounced about both the surface and deep colonies. The colonies of *Streptococcus equi* are larger, more opaque, sharply raised, and glistening. Hemolytic zones develop about both the surface and deep colonies. On horse blood agar slants the non-lactose-fermenting type produces a much thicker, glistening, opaque growth and resembles *Streptococcus mucosus*. The other forms a more delicate, thin dry film.

Preparations from the peritoneal exudate of mice dying as the result of inoculation, stained by Gram's method and counterstained with safranine, show both types grouped in pairs and short chains. A well developed capsular substance can usually be demonstrated about the cocci. In films made from the pus of infected horses *Streptococcus equi* usually appears as diplococci and chains of 10 or 12. In one instance extremely long chains were observed. *Streptococcus pyogenes* frequently occurs in pairs and in chains of 6 or 8.

DISCUSSION.

The true etiological relationship of streptococci to both strangles and influenza is still in doubt. There can be no question, however, that they play an important part in both diseases. *Streptococcus pyogenes* has been found more frequently in horses suffering from influenza than *Streptococcus equi*, although the latter species has been isolated from four typical cases. In strangles the opposite holds true, but in certain instances *Streptococcus pyogenes* has been isolated in pure culture. It is possible that nasal and pharyngeal infections depend, to a considerable degree, on a lowered resistance. Such a condition may exist either as the result of a distinct primary infection due to another organism or virus, or to a number of external causes. The proportion of infected animals following a journey appears to depend somewhat on atmospheric conditions, diminished rations, overcrowding, and the length of the journey. One fact which points to a cause or causes other than the streptococcus is the frequency

with which apparently normal horses carry virulent *Streptococcus pyogenes* on the nasal mucosa and in the throat. Such horses in this district are purchased by farmers who immediately house them with other horses on their farms. Influenza rarely develops among the resident horses. It is not uncommon, however, for an aged horse reared in the East to develop influenza or strangles after exposure to these diseases in sales stables.

It is becoming a common practice to attempt to immunize horses with killed cultures of streptococci before shipment. From this investigation it seems that these vaccines should contain both the lactose-fermenting *Streptococcus pyogenes* and the non-lactose-fermenting *Streptococcus equi.*

The horse streptococci, especially *Streptococcus pyogenes,* resemble those of human and bovine types to a certain extent. The cow strains, from mastitis, are easily separated from those of the horse by their low pathogenicity for mice and rabbits and their ability to coagulate milk. Bovine streptococci from mastitis rarely produce well marked areas of hemolysis about the surface colonies. Hemolytic zones usually develop about the surface colonies of the pathogenic human and equine strains. The pathogenic horse streptococci closely resemble those from human diseases in pathogenicity for rabbits and mice and in their hemolytic properties. Avery and Cullen (21) have shown that the hydrogen ion concentration of the bovine strains grown in dextrose broth ranges between 4.3 and 4.5, while that of the human strains is 5.0 to 5.2. These differences have held true when measured with 0.05 N sodium hydroxide. The acid production of the bovine strains from mastitis has averaged 4.4 per cent of normal acid, of those pathogenic for the horse the net production is a little lower, 4.1 per cent. The human strains, however, are considerably lower, 2.6 per cent. Thus acid production appears to serve as a ready means of differentiation between the pathogenic animal and human strains.

SUMMARY.

The lower nasal mucosa and the pharynx of thirty eastern and twenty-three western horses have been examined for streptococci.

Eight of the eastern horses carried non-hemolytic streptococci on the nasal mucosa. From the pharynx of six, non-hemolytic strepto-

cocci were cultivated. The throats of eighteen contained strains of the hemolytic type. The nasal mucosa of the eastern horses failed to show hemolytic streptococci.

Eight western horses carried non-hemolytic streptococci in the nasal passage; eight also harbored the hemolytic type. Twenty-two strains were isolated from the pharynx. Eleven were hemolytic.

Among all the non-hemolytic nasal strains those capable of fermenting mannite predominate. Those of the non-hemolytic types from the pharynx of both classes of horses may or may not ferment lactose but all do ferment either raffinose or inulin. In no instance have any of the non-hemolytic types proved pathogenic for mice.

The hemolytic strains from the nasal mucosa of the western horses were all of the *Streptococcus pyogenes* type. They were pathogenic for mice and rabbits. One strain from the pharynx of an eastern horse and eight from the throats of the western horses were of the same species. All the others corresponded closely in their fermentation reactions with non-hemolytic streptococci from the same region.

The streptococci from pathological sources were all hemolytic. They have fallen into two groups; the larger group (*Streptococcus pyogenes*) produced acid in dextrose, lactose, saccharose, maltose, milk, and salicin but failed to change the reaction of broth containing raffinose, inulin, or mannite. The streptococci of the smaller group (*Streptococcus equi*) differ only in their inability to ferment lactose or acidulate milk. Both types are pathogenic for mice. Rabbits are usually more resistant.

Streptococcus pyogenes has been isolated from eighteen of twenty-two cases of influenza, three of six cases of strangles, and from eight of nine abscesses. *Streptococcus equi* was observed in four horses suffering from influenza and five others affected with strangles. This species was also found in an abscess and associated with both rhinitis and pharyngitis.

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EXPLANATION OF PLATE 12.

FIG. 1. Plate Culture P 40. Hemolysis Type xa; 2 per cent veal infusion agar, 12 cc., defibrinated horse blood, 1 cc.; incubation 24 hours at 38°C. The inner clearer zone surrounded by the larger area of partial hemolysis is shown.

FIG. 2. A deep colony from the same culture. Note the aggregates of unhemolyzed cells in both zones. × 60.

FIG. 3. Plate Culture P 57. Hemolysis Type x; media and incubation periods identical with those described under Fig. 1. The hemolytic area is more or less hazy due to a partial dissolution of the red cells.

FIG. 4. A deep colony of Culture P 57. Clumps of red cells are visible throughout the area of hemolysis. × 60.

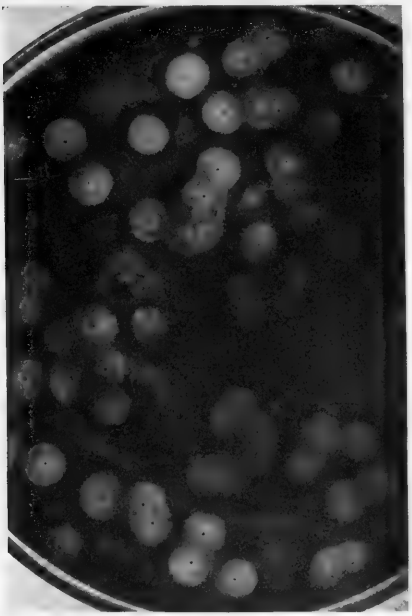

FIG. 1.

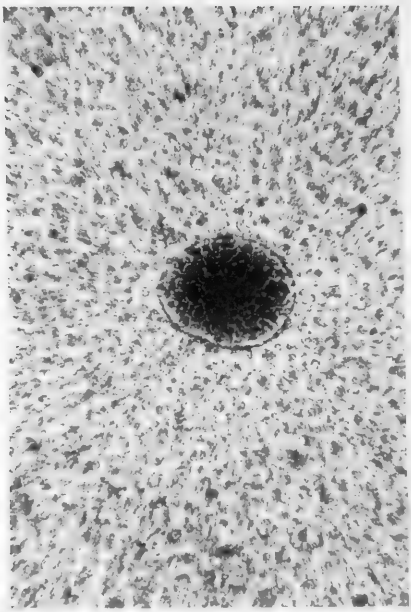

FIG. 2.

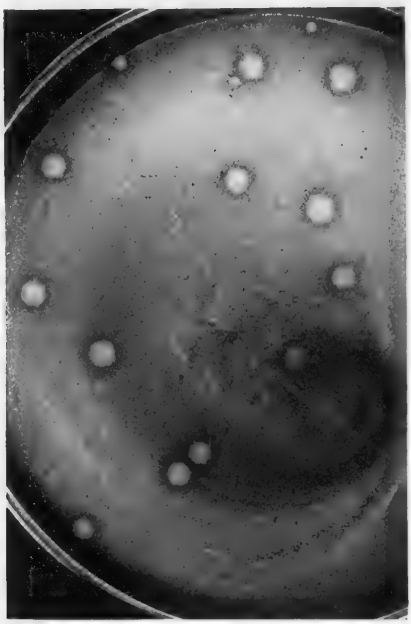

FIG. 3.

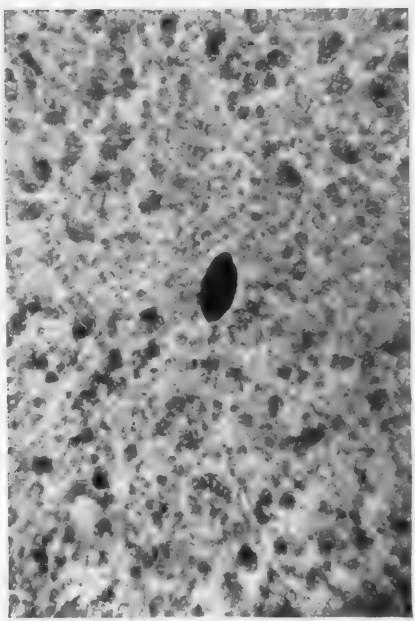

FIG. 4.

(Jones: Streptococci of equines.)

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